Nicotine vaping in England: an evidence update including health risks and perceptions, 2022

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Contents

Acknowledgements 8

Collaborators 9

Suggested citation 10

Acronyms and abbreviations 11

Conflict of interest statement 13

Executive summary 15

- Chapter 1: Introduction 15
- Chapter 2: Methods 20
- Chapter 3: Vaping among young people 21
- Chapter 4: Vaping among adults 26
- Chapter 5: Nicotine 30
- Chapter 6: Flavours 34
- Chapter 7: Biomarkers of exposure 36
- Chapter 8: Biomarkers of potential harm to health cutting across several diseases 43
- Chapter 9: Cancers 48
- Chapter 10: Respiratory diseases 51
- Chapter 11: Cardiovascular diseases 57
- Chapter 12: Other health outcomes 64
- Chapter 13: Poisonings, fires and explosions 65
- Chapter 14: Heated tobacco products 67
- Chapter 15: Harm perceptions and communications 69
- Chapter 16: Conclusions 75

1 Introduction 78

- 1.1 Objective of the report 78
- 1.2 Terminology 78
- 1.3 Vaping products 79
- 1.4 Current vaping regulations in England 79
- 1.5 MHRA safety monitoring 81
- 1.6 Medicinal nicotine vaping products 84
- 1.7 Local authority trading standards 85
- 1.8 Advertising 87
- 1.9 UK government strategies, consultations and relevant commissioned work 88

1.10 New NICE guideline 94
1.11 Selected international developments 96
1.12 Scope of this report 103
1.13 Main findings 104
1.14 Implications 106
1.15 References 108

2 Methods 114
2.1 Methods: vaping among young people and adults 114
2.2 Methods: systematic literature review of the health risks of vaping 120
2.3 Heated tobacco products use and recent evidence from a Cochrane literature review 146
2.4 Methods: systematic literature review of vaping harm perceptions 146
2.5 Overall conclusions 150
2.6 References 152

3 Vaping among young people 159
3.1 Objective 159
3.2 Surveys 159
3.3 Smoking and vaping prevalence among young people in England 160
3.4 Attempts to quit vaping 175
3.5 Effects of COVID-19 on vaping and smoking 176
3.6 Reasons for vaping 178
3.7 Order of first use of cigarettes and vaping products 184
3.8 Vaping products 186
3.9 Flavours 189
3.10 Nicotine 193
3.11 Perceived addiction and urges to vape 198
3.12 Source, place of purchase and ownership 203
3.13 Other nicotine products 208
3.14 Conclusions 209
3.15 Implications 212
3.16 References 214

4 Vaping among adults 215
4.1 Objective 215
4.2 Surveys 215
4.3 Smoking and vaping prevalence among adults in England 217
4.4 Vaping by smoking status 227

4.5 Smoking status of vapers 242
4.6 Vaping, smoking and socio-economic status 248
4.7 Reasons for vaping 254
4.8 Vaping products 256
4.9 Flavours 265
4.10 Nicotine 270
4.11 Urges to vape and smoke 274
4.12 Motivation to stop vaping 276
4.13 Other nicotine products 277
4.14 Vaping and smoking cessation in England 278
4.15 Use of vaping products for smoking cessation in England (population level data) 283
4.16 Use of vaping products in stop smoking services in England 286
4.17 Conclusions 291
4.18 Implications 293
4.19 References 294

5 Nicotine 296
5.1 Introduction 296
5.2 Evidence from previous reports on nicotine dependency 300
5.3 Evidence from previous reports on nicotine toxicity 305
5.4 Updated evidence from the systematic reviews covered in this report 309
5.5 Review of nicotine pharmacokinetic studies 310
5.6 Systematic review on exposure to nicotine and its metabolites 323
5.7 Dependency on vaping products 324
5.8 Systematic review on the health effects of vaping 328
5.9 Synthesis of animal and cell studies 329
5.10 Conclusions 332
5.11 Implications 333
5.12 References 371

6 Flavours in vaping products 383
6.1 Introduction 383
6.2 Evidence on exposure and potential harm from flavourings in vaping products 393
6.3 Findings from the systematic review 396
6.4 Conclusions 412
6.5 Implications 413
6.6 References 415

7 Biomarkers of exposure to nicotine and potential toxicants 422

7.1 Introduction 422
7.2 Summary of previous reports on vaping products use association with exposure to toxicants 422
7.3 Biomarkers of firsthand toxicant exposure 425
7.4 Biomarkers of exposure to volatile organic compounds 498
7.5 Biomarkers of exposure to tobacco-specific nitrosamines 601
7.6 Biomarkers of exposure to other potential toxicants 641
7.7 Biomarkers of exposure to carbon monoxide 667
7.8 Biomarkers of exposure to metals 696
7.9 Biomarkers of secondhand toxicant exposure 716
7.10 Conclusions 727
7.11 Implications 731
7.12 References 733

8 Biomarkers of potential harm to health cutting across several diseases 746
8.1 Introduction 746
8.2 Study characteristics 748
8.3 Risk of bias in included studies 750
8.4 Study findings 750
8.5 Conclusions 767
8.6 Implications 770
8.7 References 806

9 Cancers 813
9.1 Introduction 813
9.2 Summary of previous reports about the effect of vaping on cancer risk and outcomes 815
9.3 Findings from the systematic review 817
9.4 Conclusions 843
9.5 Implications 845
9.6 References 847

10 Respiratory diseases 859
10.1 Introduction 859
10.2 Previous reports about the effects of vaping on respiratory disease 864
10.3 Findings from the systematic review 866
10.4 Conclusions 923
10.5 Implications 926
10.6 References 928

11 Cardiovascular diseases 946
11.1 Introduction 946
11.2 Previous reports about effects of vaping on cardiovascular health and disease 947
11.3 Findings from the systematic review 949
11.4 Conclusions 999
11.5 Implications 1003
11.6 References 1006

12 Other health outcomes 1013
12.1 Introduction 1013
12.2 Summaries of previous reports 1013
12.3 Findings from the present systematic review 1015
12.4 Conclusions 1038
12.5 Implications 1039
12.6 References 1040

13 Poisonings, fires and explosions 1047
13.1 Introduction 1047
13.2 Nicotine toxicity and poisonings 1047
13.3 Fires and explosions caused by vaping products 1066
13.4 Conclusions 1078
13.5 Implications 1079
13.6 References 1081

14 Heated tobacco products 1086
14.1 Introduction 1086
14.2 HTP use among young people in England 1087
14.3 HTP use among adults in England 1089
14.4 Cochrane review: heated tobacco products for smoking cessation and reducing smoking prevalence 1092
14.5 Conclusions 1097
14.6 Implications 1098
14.7 References 1099

15 Harm perceptions and communications 1101
15.1 Introduction 1101
15.2 Methods 1101
15.3 Harm perceptions among young people 1102
15.4 Harm perceptions among adults 1116

15.5 Systematic review of vaping harm perceptions: examining interventions to change them, and longitudinal associations with vaping and smoking behaviours 1121
15.6 Conclusions 1208
15.7 Implications 1213
15.8 Appendix 1 1215
15.9 Appendix 2 1217
15.10 References 1222

16 Conclusions 1228
16.1 Preamble 1228
16.2 Regulatory structures 1228
16.3 Consumer vaping products 1229
16.4 Medicinally licensed vaping products 1230
16.5 Smokefree 2030 and vaping products 1231
16.6 International updates 1231
16.7 Nicotine vaping in England 1232
16.8 Exposure and potential health harms of vaping products 1235
16.9 General limitations of the identified literature 1236
16.10 Findings of our systematic review of exposure and potential health harms 1242
16.11 Evidence statements 1252
16.12 Overall implications 1259

Appendices 1265
Appendix 1. Table 1: risk of bias assessment of randomised controlled trials using Cochrane RoB2 tool 1265
Appendix 2. Table 2: risk of bias assessment of cross-over studies using Cochrane RoB2 tool 1267
Appendix 3. Table 3: risk of bias assessment of non-randomised longitudinal studies using ROBINS-I tool 1272
Appendix 4. Table 4: risk of bias assessment of cross-sectional studies using Biocross tool 1281
Appendix 5. Table 5: study funding sources as reported in publications 1293
Appendix 6. Table 6: a summary of in vivo (animal) studies evaluating health effects of vaping products inhalation exposure 1315
Appendix 7. Table 7: a summary of in vitro (cell) studies evaluating health effects of vaping products inhalation exposure 1388
Appendix 8. Table 8: animals and cell studies funding sources as reported in publications 1420
Appendix 9. References 1436

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Acronyms and abbreviations

Acronym	Meaning
3-HPMA	3-hydroxypropylmercapturic acid
8OhdG	8-hydroxy-2'-deoxyguanosine
APS	Annual Population Survey
ASH	Action on Smoking and Health
CO	Carbon monoxide
COPD	Chronic obstructive pulmonary disease
COT	Committee on Toxicity
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
CYMA	N-acetyl-S-(2-cyanoethyl)-l-cysteine
DNA	Deoxyribonucleic acid
EU	European Union
EVALI	E-cigarette or vaping use associated lung injury
FMD	Flow-mediated dilation
HDL	High-density lipoprotein
HTP	Heated tobacco products
IL-6	Interleukin-6
IL-8	Interleukin-8
ITC	International Tobacco Control Policy Evaluation Project
LDL	Low-density lipoprotein
MCP	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
MHBMA	Monohydroxybutenylmercapturic acid
MHRA	Medicines and Healthcare products Regulatory Agency
NAB	N-nitrosoanabasine
NASEM	National Academies of Sciences, Engineering and Medicine
NAT	N-nitrosoanatabine
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NNAL	4- (methylnitrosamino)-1- (3-pyridyl)-1-butanol
NNN	N-nitrosonornicotine
NPIS	National Poisons Information Service
NRT	Nicotine replacement therapy
OPN	Opinions and Lifestyle Survey
PG	Propylene glycol
PGE-M	Prostaglandin E2 metabolite
PHE	Public Health England
PWV	Pulse wave velocity
RCT	Randomised controlled trial
ROS	Reactive oxygen species
sICAM-1	Soluble intercellular adhesion molecule 1
sNOX2-dp	Soluble Nox2-derived peptide
SPECTRUM	Shaping Public hEalth poliCies To Reduce ineqUalities and harM
STS	Smoking Toolkit Study
TNF-α	Tumour necrosis factor alpha

TRPR	Tobacco and Related Products Regulations
TSNA	Tobacco specific nitrosamines
UK	United Kingdom
US	United States
VG	Vegetable glycerine
VOC	Volatile organic compound
WBC	White blood cells
WHO	World Health Organization

Conflict of interest statement

The authors have no links with any tobacco or vaping product manufacturers or distributors.

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Executive summary

Chapter 1: Introduction

Objective of the report

This report is the eighth in a series of independent reports originally commissioned by Public Health England (PHE) and now the Office for Health Improvement and Disparities (OHID) in the Department of Health and Social Care. The series aims to summarise the evidence on vaping products and to inform policies and regulations.

Alternative nicotine delivery devices such as vaping products can play a vital role in reducing the huge health burden caused by cigarette smoking, which remains:

- the largest single risk factor for death and years of life lived in ill-health globally
- a leading cause of health inequalities in England
- the second most important risk factor for death and disability-adjusted life years globally

Issues addressed

This current report focuses predominantly on the potential health risks of vaping. We carried out a systematic literature review of the health risks of vaping and divided the findings into chapters. These include:

- biomarkers of exposure to nicotine and potential toxicants
- biomarkers of potential harm to health cutting across several diseases, including cancer, respiratory and cardiovascular diseases
- biomarkers specifically associated with cancer, respiratory, cardiovascular or other health outcomes
- poisonings, fires and explosions
- nicotine
- flavours

This report also covers the latest evidence on prevalence and characteristics of vaping in young people and adults in England, with a focus on the data emerging since our last report published in early 2021. It looks at the prevalence of heated tobacco product use in England, incorporating a summary of the latest Cochrane Review on heated tobacco products, and a new systematic review on harm perceptions of vaping products and interventions to affect perceptions.

Our report does not cover 2 important issues. We felt these issues were either being addressed comprehensively elsewhere or had been covered in our previous reports. So, we did not examine:

The relationship between vaping and subsequent smoking. This is because a new Cochrane Review on electronic cigarettes and subsequent cigarette smoking in young people is examining the existing literature about this, among people under 30 years old.

The evidence for the effectiveness of vaping to help people who smoke quit. We have covered this topic in our previous report, and the Cochrane collaboration has an ongoing (updated monthly) systematic review on electronic cigarettes for smoking cessation. This Cochrane review examines the effectiveness of using electronic cigarettes to help people who smoke tobacco achieve long-term smoking abstinence and searches for updates of the evidence monthly.

Throughout our report, we have also tried to reflect on changes in England since our first report in 2015. This may also help to understand underlying trends, given the influence of COVID-19 recently on the availability of data and on smoking and vaping behaviours.

Terminology

As in our 2020 and 2021 reports, we use the term ‘vaping products’ to describe e-cigarettes and refill containers (e-liquids) intended for nicotine vaping. Some vaping products do not always contain nicotine. Where studies explored products without nicotine, we refer to them as non-nicotine vaping or vaping products.

We use ‘vapers’ to refer to people who regularly use vaping products and ‘vaping’ as the act of using a vaping product. These terms do not include cannabis vaping or the vaping of other legal or illegal substances, which are not the subject of this report.

Vaping regulations and guidance

Here we summarise the main regulations in England governing vaping products and their surveillance, the Medicines and Healthcare products Regulatory Agency (MHRA) safety monitoring, relevant government strategies and consultations, recent reports on

regulations, the National Institute for Health and Care Excellence (NICE) tobacco guideline, as well as selected international developments.

Main findings

Regulations and licensing

Vaping products containing nicotine are regulated under the Tobacco and Related Products Regulations 2016 (TRPR), and need to be notified to the MHRA and comply to certain standards (for example, nicotine content is limited to 20 milligrams per millilitre (mg/mL)) before they can be legally sold in the UK. An analysis of notifications in 2016 to 2017 found that notified products were unlikely to cause serious harm.

Vaping products that do not contain nicotine come under the General Product Safety Regulations 2005, enforced by local authority trading standards.

Medicinally licensed vaping products are exempt from the TRPR and currently there is no licensed product in the UK. Although, in October 2021, MHRA published updated guidance to provide clearer information on the process and help speed up review times.

Adverse advents

MHRA also collects information on adverse events believed to be associated with vaping products containing nicotine through its Yellow Card scheme. Between 20 May 2016 (implementation of TRPR) and 13 January 2022, MHRA received 257 reports of adverse reactions (26 of those since January 2021). Each report represents an individual for whom more than one adverse reaction could have been reported. A report is not proof that the reaction was caused by a vaping product, just that the reporter thought it might have been.

Since January 2021, the MHRA has considered 14 of the reports as serious and no fatalities were reported.

Adverse reactions to licensed smoking cessation medications are also reported to the MHRA. In 2021 there were 297 reports for nicotine replacement therapy (NRT) and 78 for varenicline. Varenicline has been unavailable since June 2021, further limiting effective pharmaceutical options for smoking cessation.

Age of sale

It is illegal to sell vaping products to anyone aged under 18 and to buy vaping products for anyone under 18. There is a loophole in the legislation allowing free samples of vaping products to be given to people of any age. Surveys by the Chartered Trading Standards Institute to capture tobacco control activities, including enforcement of age of sale vaping and tobacco product laws, have not been carried out since 2020.

A specific project in Scotland between October and December 2021 focused on single use disposable vaping products. It found that most products had not been notified as required with many above the 20mg/mL nicotine content limit. It also identified some violations of age of sale laws. A review of the age of sale legislation in the UK published in January 2021 concluded that overall, it had achieved its original goal of reducing uptake among under 18s.

Smokefree ambition

A government consultation in 2019 – Advancing our health in the 2020s – outlined a new ambition for England to be smokefree by 2030 (meaning only 5% of the population would smoke by then). It included an "ultimatum for industry to make smoked tobacco obsolete by 2030, with smokers quitting or moving to reduced risk products like vaping products".

The All-Party Parliamentary Group on Smoking and Health made recommendations to help achieve the smokefree 2030 ambition. These included reducing the appeal and availability of vaping products and other nicotine products to young people and to update its guidance for medicinal licensing of vaping products.

Advertising and social media

A review of vaping product marketing in the UK between 2016 and 2019 found high compliance with the advertising code in advertisements, but not in social media posts. It found that young people who had never smoked or vaped noticed posts relating to vaping more than adults who smoked. However, compared with the US and Canada, UK regulations were found to have limited exposure to marketing among adults and young people.

Recent and upcoming developments

In March 2022, OHID published the post-implementation review of the TRPR. The review assessed whether the regulations had met their objectives. This review concluded that the evidence indicated the TRPR's main objectives were being met, and provided a strong argument for retaining the regulations. It also proposed some amendments which could help support the government's smokefree 2030 ambition.

A new tobacco control plan for England will be published in 2022 and is expected to outline the government's strategy for England to become smokefree by 2030. The 2017 tobacco control plan, Towards a smoke-free generation, set out ambitions up to 2022 and remains in place, although progress towards meeting the ambitions has been mixed.

The government also commissioned an independent review of tobacco control, which was published in June 2022. It makes recommendations for the best ways to address the health inequalities caused by smoking and to achieve the smokefree 2030 ambition.

Vaping products which do not contain nicotine and are regulated through the General Product Safety Regulations 2005 are less strictly regulated than products that contain nicotine, so their regulation requires further consideration. As other non-tobacco nicotine products (such as nicotine pouches) emerge in the UK, it seems appropriate to review regulations for these products at the same time.

In November 2021, NICE published a new comprehensive guideline on tobacco, Tobacco: preventing uptake, promoting quitting and treating dependence, which includes guidance on:

- preventing uptake of smoking
- promoting quitting
- treating tobacco dependence
- discussing vaping products with patients to help prevent or stop their tobacco use

It also makes recommendations for policy, commissioning and training.

We also summarise recent international developments in vaping product policy, including in the EU, the US, Canada, Australia and New Zealand.

Implications

The smokefree 2030 ambition and developing a new tobacco control plan for England provide an opportunity to review all vaping (and other nicotine and tobacco) regulations. This will ensure that regulations are appropriate and help smokers quit, while managing the risk of uptake for people who have never smoked.

The next tobacco control plan also provides an opportunity to set out the plans needed to achieve the smokefree 2030 ambition and to set intermediate targets for smoking prevalence in different disadvantaged groups.

The continuing lack of a medicinally licensed vaping product is of concern and may require further review of the process involved.

There needs to be consideration of whether some aspects of packaging of vaping products need restricting.

The review of vaping product marketing suggests the UK needs to substantially strengthen its enforcement of marketing regulations on social media.

There is an opportunity to standardise the notification processes using the MHRA database of notified vaping products. This would enable research and help to maximise harm reduction potential.

Local authority trading standards efforts have been scaled down and compliance with regulations is not enough to prevent underage sales and access to illicit products. Also, more frequent surveillance of single-use disposable vaping products is needed. There is a danger that the reduction in local trading standards officers and restructure of the MHRA could result in a lack of surveillance of these products. This could undermine the approach and regulatory framework for vaping products adopted in England.

Lessons should be learned from the mislabelled US 'e-cigarette, or vaping, use-associated lung injury' (EVALI) outbreak. These lessons include the impact of miscommunications about nicotine vaping compared to vaping contaminated illicit substances. Communications about EVALI should clearly separate vaping these illicit substances from nicotine vaping. Also, communications about any future cases or outbreaks of poisonings or injuries should be clear about the implicated substances.

Chapter 2: Methods

We used data from 2 surveys for information on smoking and vaping among young people in England. These were the:

- Action on Smoking and Health-Youth (ASH-Y) survey
- International Tobacco Control Policy Evaluation Project (ITC) Youth Tobacco and Vaping survey (hereafter known as ITC Youth survey)

For information on smoking and vaping among adults in England, we used data from 4 surveys, which were:

- ONS Annual Population Survey (APS)
- Smoking Toolkit Study (STS)
- Action on Smoking and Health-Adult (ASH-A) survey
- ONS Opinions and Lifestyle Survey (OPN)

We reported NHS Digital data from stop smoking services on supported stop smoking attempts in England, and from the National Poisons Information Service and London Fire Brigade on suspected poisonings and fires caused by vaping products.

We conducted 2 systematic reviews, one on the health risks of vaping and one on vaping harm perceptions and also summarised findings from a recent Cochrane Review on heated tobacco products.

For chapters on vaping associations with health risks, we first summarised evidence from previous reports by PHE, the National Academies of Sciences Engineering and Medicine (NASEM) and the Committee on Toxicity of Chemicals in Food Consumer Products and the Environment (COT). We then presented findings from our systematic review. To summarise evidence on the health risks of vaping, we developed an algorithm to assess whether to conduct meta-analyses. Details of the algorithm are presented in table 6 in chapter 2 of the full report.

Chapter 3: Vaping among young people

Data collection

Data reported in this chapter were collected in February 2021 (from the ITC Youth survey), in March to April 2021 (from the 2021 ASH-Y survey) and we also report prevalence data from the ASH-Y 2022 survey carried out in February to March 2022.

In response to the COVID-19 pandemic, schools were closed in England between 4 January and 15 March 2021, and there were tight restrictions on social gatherings between 4 January and 19 May 2021. Although no restrictions were in place during 2022 data collection, it is likely that there are ongoing effects of the 2 years of social restrictions on young people. So, conclusions in this chapter may be greatly affected by the COVID-19 regulations.

Main findings

Smoking and vaping prevalence

2022 ASH-Y survey data (11 to 18 year olds) showed:

- smoking prevalence (including occasional and regular smoking) was 6.0% in 2022 (compared with 4.1% in 2021 and 6.7% in 2020)
- vaping prevalence (including occasional and regular vaping) was 8.6% in 2022 (compared with 4.0% in 2021 and 4.8% in 2020)

2021 ITC Youth survey data (16 to 19 year olds) showed:

- smoking prevalence (defined as smoking more than 100 cigarettes in their life and having smoked in the past 30 days) was 7.9% in February 2021 (compared with 8.5% in February 2020 and 6.2% in August 2019)

- vaping prevalence (defined as vaping on more than 10 days in their lifetime and having vaped in the past 30 days) was 9.1% in February 2021 (compared with 9.4% in February 2020, and 7.7% in August 2019)

Overall, data from the 2021 ASH-Y and ITC Youth surveys were broadly similar for comparable age categories. Vaping among 19 year olds has been steadily increasing in the ITC Youth data over recent years.

The 2022 ASH-Y data suggests that overall nicotine use (via smoking or vaping) has increased over the past year, being 11.1% in 2022 compared with 6.2% in 2021. In 2015, the proportion was 7.7%.

Based on the socio-economic grade of 11 to 18 year olds in the 2022 ASH-Y survey, the estimates for smoking and vaping prevalence were similar for the more advantaged groups in social grades A, B and C1 (5.8% for smoking, 8.4% for vaping) to more disadvantaged groups in social grades C2, D and E (5.4% for smoking, 8.1% for vaping). This was a departure from previous years. For example, in 2021, the estimates for smoking and vaping prevalence were higher among the more advantaged groups in social grades A, B and C1 (4.6% for smoking, 4.4% for vaping) than for the more disadvantaged groups in social grades C2, D and E (2.8% for smoking, 3.0% for vaping), similar to ASH-Y data from previous years.

The 2022 ASH-Y data showed that most young people who had never smoked were also not currently vaping (98.3%). This was consistent with the 2021 ASH-Y and 2021 ITC Youth data, although the proportions were higher (99.2% and 99.1% respectively).

Vaping devices

Disposable models (which are pre-filled with liquid and used only once) were the most popular type of vaping device in the 2022 ASH-Y survey. These were used by 52.8% of 11 to 18 year olds who currently vaped, and 18.7% used tank models (which are reusable and rechargeable kits that users can refill with liquid). This was a stark difference from previous years, where tank models were the most popular type of vaping device. For example, in 2021, only 7.8% of current vapers reported using disposable models, whereas 41.0% used tank models.

COVID-19

Young people from the 2021 ITC Youth survey reported an effect of COVID-19 on smoking and vaping behaviour, which found:

- 8.0% of past year vapers reported quitting vaping
- 15% of past year vapers reported cutting down due to the COVID-19 pandemic

However, 15% reported vaping more as an effect of the pandemic. Similar patterns were seen among young people who had smoked in the past year, with:

- 7% reporting quitting
- 20% reporting cutting down
- 18% reporting smoking more

These findings could contribute to the slight increase in former smokers (from 0.8% to 1.7%) and former vapers (from 4.6% to 8.6%) observed in the ITC Youth data between 2019 and 2021.

Reasons for vaping

The main reasons for vaping were to “give it a try” (48.8%, 2021 ASH-Y), and “liking the flavours” (37.2%, ITC Youth). These reasons were most common among young people who have never smoked or only tried smoking. Among young people who smoked, or had smoked, in the ITC Youth survey, harm reduction, and quitting related reasons were common.

In the 2021 ASH-Y survey, most 11 to 18 year olds who had tried vaping had smoked first (38.7%), while 24.7% said they had vaped before they smoked and 29.7% said they had tried a vaping product and never tried smoking.

Flavours

Fruit flavours were the most popular among young people who currently vaped (51.5% in 2021 ASH-Y). This was followed by “menthol/mint” (13.0%), then “chocolate/dessert/sweet/candy" flavours (9.3%), similar to data presented in our 2021 report.

Access to vaping products

Although it is illegal to sell vaping products to under 18 year olds, many young people under the age of 18 bought and owned their own vaping devices. In the 2021 ASH-Y

survey, just under a quarter (24.8%) of young people aged 11 to 17 said that they were given products by friends. But others also reported buying them, for example:

- 22.1% said they bought them from newsagents
- 22.1% said they bought them online
- 16.3% said they bought them from a supermarket

Similarly, in the ITC Youth survey, young people aged 16 to 17 who had vaped in the past 30 days commonly reported being given products (37.5%). Many also reported buying products from shops (32.1%) or online (23.3%). Nearly two thirds (64.3%) of 16 to 17 year olds from the ITC Youth survey who had vaped in the past 30 days reported they owned a vaping product.

Nicotine

About a third (34.2%) of 11 to 18 year olds in the 2021 ASH-Y survey who currently vaped or had vaped in the past reported always using vaping products that contained nicotine and 20.4% reported always using nicotine-free products. Just over two-thirds (68.9%) of 16 to 19 year olds who had vaped in the past 30 days and had ever used vaping products with nicotine, reported using nicotine in their current vaping product and 12.3% said their vaping product did not contain nicotine.

In the 2021 ITC Youth survey, the most common nicotine strength used by 16 to 19 year olds who had vaped in the past 30 days was reported to be under 20mg/mL (64.0%). A total of 17.2% reportedly used a strength between 20 mg/mL and 49 mg/mL and 5.6% reportedly used 50 mg/mL or over. Compared to 2019, fewer participants reported they did not know the strength of their vaping liquid (from 19.6% to 7.3%).

About half (53.1%) of 16 to 19 year olds who vaped in the past 30 days reportedly used nicotine salt e-liquid (a nicotine version which is smoother to inhale, has lower pH and is absorbed faster into the bloodstream than freebase nicotine) similar levels to those seen in 2019 (56.6%). We also found that 40.4% did not use nicotine salts and 6.5% were unsure. This has changed compared to 2019, where 30.6% did not use salts and 12.8% were unsure. Overall, there was higher awareness of the inclusion of nicotine and type of nicotine (freebase or salt) and fewer “don’t know” responses in 2021 compared to 2019.

Perceived addiction and urges to vape

Under half (42.8%) of 16 to 19 year olds in the 2021 ITC Youth survey who currently vaped did not feel addicted to vaping, but half (52.5%) said they felt a little or very addicted. In comparison, 14.5% of 16 to 19 year olds who currently smoked did not feel addicted to smoking, and 83.0% reported they felt a little or very addicted.

Just under half (44.5%) of 16 to 19 year olds in the 2021 ITC Youth survey who currently vaped reported experiencing urges to vape almost daily or more than daily, and 16.8% reported never experiencing an urge to vape. In comparison, 66.6% of young people who currently smoked reported urges to smoke daily or multiple times a day, and only 4.7% reported never having urges to smoke.

Just over forty per cent of 11 to 18 year olds in the 2021 ASH-Y survey who currently vaped said they did not feel any urges to vape at all (41.5%), and 23.5% reported strong, very strong or extremely strong urges to vape. In comparison, 24.3% of those who currently smoked reported no urge to smoke and 31.4% reported a strong, very strong or extremely strong urge to smoke.

Other tobacco and nicotine products

Just over one-tenth (11.0%) of 16 to 19 year olds in the ITC Youth survey reported ever using a waterpipe, 4.0% reported ever using nicotine pouches, and 5.0% reported ever using smokeless tobacco.

Implications

Further monitoring and research

Dependence on vaping assessed in 2021 appears lower than on smoking for young people. Further research on dependence is needed, including dependence by type of vaping product used, nicotine type and nicotine strength.

Vaping and smoking among young people appear to have decreased between 2020 and 2021 but then increased in 2022. So, it is important that trends continue to be monitored by the government. The differences in estimates between the ASH-Y and ITC Youth surveys in 2021 are likely due to differences in the age groups and a higher prevalence of vaping among 19 year olds who are included in the ITC Youth but not the ASH-Y. There are also possible lasting effects of the COVID-19 pandemic and its impact on smoking and vaping among young people needs to be monitored.

Enforcement and further regulations

In 2022, higher vaping prevalence was reported across all age categories. So, as recommended in our previous reports, enforcement of age of sale regulations for vaping (and smoking) needs to be improved to reduce young people’s access to vaping products and cigarettes.

The dramatic increase in young people using disposable vaping products should be monitored with improved regulatory oversight. Also, the advertising, packaging and

marketing of disposable products to young people should be investigated and, where appropriate, proportionate action taken to reduce appeal to young people.

Chapter 4: Vaping among adults

Data reported in this chapter came from 4 different surveys. Most data were from the Smoking Toolkit Study (STS), collected between January and September 2021, and the 2021 ASH-Adult (ASH-A) survey, collected in February and March 2021. Other data from the ONS Opinions and Lifestyle Survey (OPN) and ONS Annual Population Survey (APS) were collected in 2020. We also report some data from the most recent 2022 ASH-A survey on smoking prevalence, vaping prevalence, the relationship between smoking and vaping and the type of vaping products used.

Main findings

Smoking and vaping prevalence

Smoking prevalence among adults in England in 2021 was between 12.7% and 14.9% depending on the survey and in 2022, based on ASH-A data, 13.2%. These equate to between 5.6 and 6.6 million smokers.

There was variation in smoking prevalence by age, gender, socio-economic status and ethnicity. Most notably, smoking prevalence remained significantly higher among adults from more disadvantaged groups.

Vaping prevalence among adults in England was lower than smoking prevalence across all groups and seemed to have increased by around 1 percentage point from 2020 to 2021, to between 6.9% and 7.1%. This equated to about 3.1 to 3.2 million vapers. In 2022, based on ASH-A data, adult vaping prevalence in England was 8.3%.

There was some variation in vaping prevalence by socio-demographic groups and smoking status. Using 2021 STS data, the highest vaping prevalence was among:

- men (7.8%)
- people from the north of England (8.3%)
- people from social grades C2, D and E (8.8%)
- current smokers (22.0% compared with 11.6% among former smokers and 0.6% among never smokers)

Among former smokers, 27.9% of short-term former smokers (quit for less than one year) used vaping products, compared with 9.9% of long-term former smokers (quit for longer than one year). This is an increase since 2013 when 1.2% of long-term former smokers vaped. In comparison, a small but steady proportion of long-term former smokers have used NRT (around 2% to 4%) since 2013.

The proportion of vapers who also smoke had been declining since 2012, from 91.9% to 49.8% in 2020 in the STS survey and from 73.7% to 31.0% in 2021 in the ASH-A survey. However, both STS and ASH-A surveys suggest a recent increase in the proportion of vapers who smoke. The STS survey showed an increase to 51.7% in 2021, and the ASH-A survey showed an increase to 33.4% in 2022. The discrepancy in estimates across surveys is likely due to different definitions of smoking status.

Types of vaping device

In both STS and ASH-A surveys, tank models remained the most popular type of vaping device, used by 59.3% of current vapers in the 2021 STS survey and 64.3% of current vapers in the 2022 ASH-A survey. In the 2021 STS survey, different types of vaping devices reported by current vapers included:

- 20.1% modular vaping products (where people use their own combination of device parts)
- 14.9% cartridge models (a rechargeable vaping device that uses replaceable pre-filled cartridges)
- 4.6% disposables (a non-rechargeable and non-refillable vaping device)

The 2022 ASH-A survey showed higher use of disposable vaping products than in 2021, with 15.2% of current vapers reporting using disposable vaping products in 2022 compared with 2.2% in 2021.

Vaping frequency

Among adults who had ever vaped, daily vaping was associated with their smoking status. Among never smokers who had ever vaped, nearly two-thirds (64.9%) had tried it once or twice and 5.0% were vaping daily. Among current daily or non-daily smokers who had ever vaped, around 27% vaped daily. Among former smokers who had ever vaped, more than half (57.7%) vaped daily (2021 ASH-A).

Length of time vaping

ASH-A 2021 data suggested an increase in the proportion of current vapers who have vaped for more than 3 years (23.7% in 2018, 29.3% in 2019, 39.2% in 2020 and 43.7% in

2021). People who had vaped in the past mostly stopped after 6 months of use or less (57.2% in 2021).

Reasons for vaping

The most common reasons for vaping reported in the 2021 ASH-A survey were to quit (27.9%) or stay off (17.7%) smoking tobacco or because people enjoyed it (12.6%).

Nicotine

In 2021, strengths of nicotine vaping liquids above those allowed by regulations (more than 20 mg/mL) were used by less than 6% of vapers. Just over a third of vapers (34.0%) reported reducing the strength of the nicotine vaping liquid they use since starting to vape, 31.4% continued using the same strength and 26.2% did not know if they had changed the strength. Just 8.1% of people reported having increased the strength of the nicotine in vaping liquid they use since starting to vape (2021 ASH-A). The proportion of vapers unsure about the strength they are using has increased slightly over the last 2 years.

Flavours

Fruit (35.3%), menthol/mint (22.5%) and tobacco (20.9%) remained the most popular flavours among vapers (2021 ASH-A).

Using vaping to stop smoking

Attempts to stop smoking and success rates for adults who tried to stop smoking increased significantly in the last 2 years. This is most likely due to the COVID-19 pandemic. According to STS data, vaping products remained the most common aid used in a quit attempt.

The 'Reaching Out' report from ASH has shown that stop smoking services have greatly improved the provision of vaping products to support a quit attempt. In 2019, 11% of surveyed local authorities offered vaping products to some or all smokers accessing stop smoking services. In 2021, 40% of surveyed local authorities offered vaping products to some or all smokers and a further 15% had plans to do so.

Between April 2020 and March 2021, quit attempts in stop smoking services that involved using a vaping product (alone or in combination with medication) achieved self-reported short-term success rates of 64.9%, compared with 58.6% for attempts not involving a vaping product. Despite this, only 5.2% of quit attempts supported by a stop smoking service involved a vaping product.

Implications

Further monitoring and research

Vaping is more common among disadvantaged adult groups in society. This mirrors smoking prevalence, and research should continue to explore the impact that higher vaping prevalence has on stopping smoking and reducing health inequalities.

The continuing impact of COVID-19 on smoking and vaping among adults needs to be monitored. This should include younger adults who start smoking and vaping and any changing patterns in the data.

There needs to be further research into the increasing proportion of long-term vapers and their motivation to stop vaping, and whether people who want to stop vaping need support. More research is also needed into vaping among:

- never smokers
- younger adults
- people from ethnic minority backgrounds

A recent increase among these groups of using disposable vaping products warrants further monitoring and research.

Implementing NICE guidance

The NICE guidance Tobacco: preventing uptake, promoting quitting and treating dependence should encourage more stop smoking services to support smokers who want to stop smoking with the help of a vaping product.

As we recommended in previous reports in this series, and as supported by the new NICE guidance, all smokers should be supported to stop smoking completely, including dual users who smoke and vape.

Chapter 5: Nicotine

In this chapter we discuss the role of nicotine in vaping product use.

Main findings

E-liquids

As discussed in chapters 3 and 4, 2021 survey data from England shows that nicotine would appear to play an important driver of adult vaping, but perhaps less so than for tobacco smoking.

Most adults who vape (about 87%) use vaping products that contain nicotine. The proportion was about 70% for 11 to 18 year olds, with about half of those saying that their vaping products always contained nicotine, and half sometimes contained nicotine. Among 16 to 19 year olds who reported ever using vaping products with nicotine, and who had vaped in the past 30 days, 83% said that their products contained nicotine or that some of their products contained nicotine. Overall, the vast majority were using vaping products with less than 20 mg/mL nicotine e-liquids and so complied with current vaping product regulations.

Questions on the use of salt-based nicotine products as opposed to freebase nicotine were not often included in surveys. Among 16 to 19 year olds, there was higher awareness of the inclusion of nicotine and type of nicotine in 2021 compared to 2019. Among adults, uncertainty about whether people who vape were using salt-based vaping products had increased slightly over the last 2 years.

Nicotine intake

Previous reviews showed that nicotine intake from vaping products was variable and dependent on different product characteristics. The updated evidence presented here also provides conclusive evidence of this variability.

The updated evidence from pharmacokinetic studies (studies exploring how nicotine is absorbed, distributed and eliminated from the body) on vaping show that in general, vaping products provide lower peak nicotine levels and lower overall nicotine levels to users than smoking provides. Also, the pharmacokinetic studies show that exposure to nicotine from vaping varies by product characteristics. The studies suggested that exposure to nicotine tends to increase when:

- using e-liquids with higher nicotine concentration
- using e-liquids based on nicotine salts rather than freebase nicotine

- using tank or modular type vaping devices which provide more exposure than cartridge or disposable models

- people with longer vaping experience vape, as they have more effective puffing behaviour

The time taken to reach peak nicotine delivery from vaping products is usually slower compared with smoking a cigarette. But this varies depending on the e-liquid nicotine concentration and the type of vaping device. Flavours may also play a role in nicotine delivery and we review this in chapter 6 on flavours.

The pharmacokinetic studies are consistent with the studies discussed in chapter 7 on biomarkers of exposure to nicotine and potential toxicants which generally showed lower exposure to nicotine when using vaping products over the short term (up to 7 days) compared to smoking. However, there was moderate evidence, in medium to longer term studies (up to 2 years), of similar exposure to nicotine from vaping compared to smoking. For experienced adult vapers, there was substantial evidence of comparable exposure to nicotine from vaping and smoking. There was supportive evidence that over time, people who vape compensate for lower nicotine concentrations by compensatory puffing (such as puffing more frequently, puffing larger volumes of aerosol, or taking longer puffs).

Nicotine dependency

There was substantial evidence from previous reports (from NASEM, COT and our 2018 report) that using vaping products can result in symptoms of nicotine dependency. There was moderate evidence that the risk and severity of nicotine dependency for vaping is lower than for cigarette smoking and would vary by product characteristics. The pharmacokinetic studies reviewed are consistent with this.

Our review showed that there are many scales used to assess nicotine and vaping dependency. But as yet, there is no consensus on which is the best scale to assess vaping dependency. So, this makes assessing the risk and severity of vaping dependency compared to tobacco smoking dependency difficult.

A recent systematic review examining the effects of nicotine concentration and flavours on dependency found that higher nicotine concentrations might increase the abuse potential and appeal of vaping and hence dependency. So, this could help someone completely substitute tobacco cigarettes for vaping products. Also, preliminary evidence suggested that flavours may interact with nicotine concentrations to affect abuse potential.

Health risks

We review the health risks of vaping in chapters 8 to 12.

Isolating the effects of nicotine on health risks in human studies is complex, partly because only a small proportion of people vape non-nicotine products. In general, where studies assessed biomarkers in humans (measurements of potentially harmful smoking or vaping effects in the body) through non-nicotine vaping as well as nicotine vaping, the different methods used in each study made it difficult to compare, and so limited our conclusions.

One biomarker, pulse wave velocity (which measures blood pressure pulse through an artery or arteries), did seem to be affected by nicotine in vaping products, at least in acute exposure studies. Evidence from the reviewed animal and cell studies suggest some adverse effects of nicotine, but the extent to which these findings can be generalised to humans is currently very unclear.

Implications

Improved surveillance and further research

Questions in national surveys sometimes lag behind product developments, such as questions about people using salt-based vaping products or increasing their use of disposable vaping products. Having an appropriately resourced product surveillance system would help to ensure researchers can capture data on product developments.

Exploring how nicotine labelling could be improved could also be useful as there appears to be an increase in adult users not knowing how much nicotine was in their vaping products. It would also be useful to further explore the small proportion of adults who use nicotine-free vaping products. For example, asking them how long and how often they use these products.

Current evidence shows that more experienced vaping product users adjust their puffing behaviour to attain higher levels of nicotine. However, this does not compensate for lower overall nicotine exposure after a single vaping session compared with smoking a cigarette. We found during longer-term vaping sessions or where a person can vape as much or as often as they want to (ad libitum), experienced vapers reach levels of nicotine comparable to those from smoking (as shown by nicotine biomarker data). Vapers' ability to adjust their puffing behaviour to mirror smoking suggests that vaping enables users to carefully control their nicotine levels. This could be a problem when people using vaping products with lower nicotine concentrations compensate by increasing their puffing and so risk increasing exposure to other constituents, including potentially harmful ones. We explore this issue further in later chapters. A recent systematic review suggested that limiting nicotine concentrations in vaping products might reduce smoking cessation.

Future research should use more longitudinal study designs (studies that assess the same people more than once over a period of time) to explore how, with more experience, vapers change their:

- puffing behaviour
- nicotine intake
- dependency, over time

This is important for people who have smoked as well as those who have never smoked. For people who have never smoked and start using nicotine through vaping, measurements are needed across a range of vaping products and their characteristics. This will help to assess whether higher nicotine limits (more than 20 mg/mL) affect a person's dependency on vaping, and how their vaping behaviour might interact with:

- free-base or salt nicotine levels
- flavours
- other characteristics (for example e-liquid PG/VG ratio)

Research on longer-term vaping behaviour would also allow researchers to clarify how using different nicotine strength e-liquids over time is associated with dependency and potential health risks.

Need for global standards and protocols

Having a global consensus for assessing and measuring nicotine and product dependency would enable comparisons of nicotine and product dependency:

- between vaping and smoking
- across different vaping products
- with different groups of users (such as adults and young people).

In England, it is important for researchers to keep up to date on the ongoing research in this area.

Agreeing a standard protocol for vaping product pharmacokinetic studies would also enable meaningful comparisons across different vaping products and e-liquid characteristics. However, more long-term ad libitum pharmacokinetic studies are also needed to reflect how users' personal experience and puffing behaviours affect nicotine delivery and dependency.

Isolating the risks of nicotine to health from the risks of other vaping constituents is difficult in human studies compared to animal and cell studies. Having standards, particularly for human cell research, may strengthen how widely or generally applicable such studies are to vapers. Such standards would also be beneficial in helping to examine the effect of nicotine in humans.

Chapter 6: Flavours

This chapter:

- describes the use of flavoured vaping products in England
- provides an overview of the role of flavours in vaping product use
- summarises the evidence on potential harm from flavourings in vaping products from studies identified in a systematic review

Main findings

Use of flavours

As we identified in earlier chapters, fruit flavours are the most popular e-liquid among adults and young people who vape in England, followed by "menthol/mint". There is some evidence to suggest that non-tobacco flavours, particularly sweet flavours, may play a positive role in helping people switch from smoking to vaping.

A systematic review of the evidence on youth use of e-liquid flavours concluded that existing research does not yet provide a clear understanding of how flavours in vaping products are associated with young people taking up or stopping smoking.

Potential toxicants in flavours

In 3 studies, levels of tobacco specific nitrosamines and volatile organic compounds were significantly reduced in smokers and dual users who switched to vaping products with different flavours. Biomarker levels slightly differed between flavours, but this was not tested for statistical significance. Users of fruit-only flavoured vaping products had significantly higher concentrations of a biomarker for acrylonitrile (CNEMA) compared to users of a single other flavour in one study.

One longitudinal observational study of people who vaped found that:

- flavour preferences changed over time
- 6.9% self-reported an adverse reaction that they associated with the flavour they used

- a third had ever used a cinnamon or cinnamaldehyde containing vaping product

Findings from 13 cell and 9 animal studies suggest there is limited evidence that some flavourings in vaping products, particularly cinnamaldehyde, or buttery or creamy flavours have the potential to alter cellular responses but less than exposure to tobacco smoke. Exposure to propylene glycol or vegetable glycerine (PG/VG) base liquids without added flavourings appeared to have little or no effect. It was not always possible to differentiate the effect of nicotine or solvents from flavourings due to lack of appropriate controls. This was further complicated by variability of e-liquid composition, cell types, dose exposure and duration. Also, there was not a great deal of consistency about whether cells or animals were exposed to e-liquids, aerosol extracts or aerosols.

There was only one study that looked at the stability of e-liquid flavourings over a period of one year (and found they were stable), but no studies conducted assessments to see if this changed how the flavouring tasted and felt over time.

Subjective effects

Two studies assessing acute exposure to flavoured vaping products, under controlled conditions, found that nicotine delivery and 'positive subjective effects' (such as liking) for flavoured vaping products were lower than for tobacco cigarettes. The studies also found that positive subjective effects were greater for vaping products and tobacco cigarettes, than for nicotine gum. There were mixed findings on whether or not the subjective effects of flavourings were due to nicotine delivery or increased level of consumption.

A recently published systematic review concluded that flavours affected the abuse potential (for example, liking a product and intending to use it again) of vaping products through increasing product appeal. But it acknowledged that the effect of flavours on smoking cessation needed further research.

Implications

Surveys in England should include detailed questions on the use of flavours (including mixing different flavours) in vaping products annually, to track use over time. Longitudinal data in adults and young people in England would also be helpful in assessing the health effect of flavours in vaping products.

The findings of the systematic review support previous reports, our 2018 report, the NASEM report and the COT review, which suggest cinnamaldehyde-containing vaping products continue to be a cause of concern. The review also recommends that regulatory bodies should review this flavouring chemical in e-liquids. Although there is less evidence in this systematic review, some in vitro (laboratory cell-based) studies suggest buttery and creamy flavoured e-liquids may also require further review.

A more standardised approach is needed to evaluate the risks associated with flavourings in e-liquids and aerosols in human and cell studies, independent of nicotine and PG/VG. The evaluation framework devised by COT to aid risk assessment of flavouring compounds via inhalation exposure could be considered by regulators at the time of product notification.

COT also suggested that since flavourings can undergo thermal degradation or react with other constituents in e-liquids, research is needed to fill the gap in our knowledge about how heating affects flavours. This included looking at the extent to which thermal degradation may be affected by users customising their vaping devices.

COT also suggested looking at the potential safety of exposure to mixing e-liquid flavours.

Also, further research is also needed about the stability of flavourings over time and whether they degrade or not.

Chapter 7: Biomarkers of exposure

Evidence reviewed

This chapter examined findings from our systematic review on biomarkers of nicotine and potential toxicants (chemicals or their metabolites in a body that show actual human exposure to nicotine or tobacco products) relevant to our 2 review protocol questions:

1. The effect of vaping and secondhand exposure to vaping products that are associated with the risk of health conditions.

2. The effects of vaping among people with existing health conditions on disease outcomes.

However, we did not find a study addressing the second review question. Only one study assessed participants with self-reported respiratory symptoms but did not test for statistical differences across relevant groups. So, our review for this chapter is confined to our first review question.

We assessed both relative (between vapers and smokers) and absolute (between vapers and non-users) vaping risks associated with exposure to nicotine and potential toxicants where the data were available. Where feasible, we included comparisons across different population groups.

The included studies used a range of different designs and had varying quality or risk of bias.

The studies we have included used a range of different definitions of vaping and smoking. For example, findings of some studies were confounded by classifying vapers who smoke, occasional vapers and/or exclusive daily vapers as a uniform group, or comparing occasional vapers with daily smokers. So, findings need to be cautiously interpreted.

Studies looking at participants at more than one time point mostly explored acute exposure to vaping (single use to 7 days) or followed up participants for short to medium term (8 days to 12 months). So, we were unable to summarise findings on longer term (more than 12 months) vaping exposure, with some studies not allowing adequate wash-out periods for biomarkers with longer half-lives.

In line with our algorithm (chapter 2, table 6), we carried out meta-analyses wherever possible. But a lack of consistency in study designs, biomarker reporting, group definitions and exposure periods resulted in only a few studies being included.

Here we summarise our findings for each biomarker for relative and absolute differences in various populations of interest, starting with first-hand vaping exposure.

Main findings

Nicotine

There was substantial variation across the 60 studies included in this section looking at nicotine exposure. Only 5 studies (4 longitudinal and one cross-sectional (measured at a single point in time)) were from the UK. Levels of nicotine and nicotine metabolites in participants using vaping products differed according to:

- study design
- definitions of vaping and smoking
- biomarker and biosample (a biological sample, which could include urine, blood plasma, blood serum and saliva) used
- exposure duration

To assess relative exposures between vaping and smoking, we were able to carry out 5 meta-analyses of nicotine and nicotine metabolites (one longitudinal, 4 cross-sectional) among people who vaped and smoked at least weekly. All found no significant differences between people who vaped and smoked.

From the narrative summaries, evidence suggests that over time and with increased experience of vaping, users can derive similar levels of nicotine as they can from smoking

cigarettes. Levels of nicotine metabolites varied with vaping device characteristics (for example, vaping device types, e-liquid nicotine concentrations).

To assess absolute exposures between vapers and non-users, we were able to carry out 4 meta-analyses of nicotine biomarkers which, as expected, showed significantly higher levels among vapers than non-users. In general, findings from the narrative summaries were similar for absolute nicotine exposures.

There were no discernible differences between adults and adolescent exposures to nicotine and its metabolites.

Volatile organic compounds

Twenty-four studies assessed volatile organic compounds (VOCs), with only 5 from the UK. VOCs are potentially harmful gases released into the air, for example while smoking tobacco. Again, there was considerable variation across the studies in:

- design
- definitions of vaping and smoking, biomarker measurements
- exposure duration

To assess relative exposures between vaping and smoking, we were able to carry out 15 meta-analyses of VOCs (4 longitudinal, 11 cross-sectional). Findings varied by biomarker. In general, most findings showed statistically significantly lower levels of VOCs among vapers than smokers, with substantial reductions in some biomarkers, such as the acrolein metabolite 3-HPMA (71%), the acrylonitrile metabolite CNEMA (94%) and 1,3-Butadiene metabolite MHBMA (83%). For a few VOCs, such as formaldehyde and toluene, available evidence was inconclusive on the significant differences between vapers and smokers.

To assess absolute exposures between vapers and non-users, we were able to carry out 10 meta-analyses (all cross-sectional). All showed no significant differences between vapers and non-users, except for the acrylonitrile metabolite CNEMA. One study showed that average levels of acrylonitrile metabolite CNEMA for vapers were over 3 times higher than those among non-users.

In general, findings from the narrative summaries were similar for absolute and relative VOC exposures.

Levels among young people were broadly in the same direction to levels reported among adults, with some differences for individual biomarkers. This may be due to different smoking and vaping patterns.

Tobacco specific nitrosamines

Twenty-eight studies assessed tobacco specific nitrosamines (TSNAs), a group of chemicals found in tobacco and tobacco smoke, some of which are harmful and cause cancer. Only 3 studies were from the UK. As with other biomarkers, there was considerable variation across the studies in:

- design
- definitions of vaping and smoking
- biomarker measurements
- exposure duration

To assess relative exposures between vaping and smoking, we were able to carry out 5 meta-analyses of TSNAs (2 longitudinal, 3 cross-sectional). These all showed significantly lower levels of TSNAs among vapers than smokers, with substantially lower levels for NNAL (58%), NAB (87%), NAT (94%) and NNN (90%). Findings were generally consistent with those reported in the narrative review.

To assess absolute exposures between vapers and non-users, we were able to carry out 3 meta-analyses using cross-sectional data. These all showed significantly higher levels of TSNAs among vapers than non-users. However, the cross-sectional data make it difficult to distinguish exposure from vaping products from previous tobacco use. Also, evidence from a randomised control trial (RCT) and a cross-over study (a study where different products are given to the same participants but in different orders, and participants serve as their own controls) indicates that TSNA metabolite levels among vapers might decrease to a similar level as among non-users.

Levels among young people were in the same direction as among adults, although the magnitude of difference between vapers and smokers was substantially less for young people compared with adults. Again, this may be due to different smoking and vaping patterns among adults and young people.

Other potential toxicants

Nine studies assessed a range of other potential toxicants, such as polyaromatic hydrocarbons, with only one from the UK. We were unable to carry out any meta-analyses. Generally, the very limited findings suggested the levels of these other potential toxicants were lower among vapers than smokers, and higher among vapers than non-users.

Carbon monoxide

Thirty-three studies assessed carbon monoxide (CO) exposure, with 3 studies from the UK. As for other biomarkers, there was considerable differences in methods across the studies and user definitions.

To assess relative exposures between vaping and smoking, we carried out 2 meta-analyses. Both showed significantly lower blood carboxyhaemoglobin levels among vapers than smokers.

We were unable to carry out any meta-analyses of exposures between vapers and non-users. But some interventional studies (such as RCTs, longitudinal and cross-over studies) suggested that exposure to CO in smokers who completely switch to vaping product use might be reduced to levels similar to non-users.

Metals

Ten cross-sectional studies examined a range of metals (arsenic, cadmium, lead, mercury), with none from the UK. No meta-analyses could be carried out.

In general, the studies had mixed findings about relative exposure.

Absolute exposure assessments were also mixed although most studies showed higher levels of exposure among vapers than non-users.

Secondhand exposure

Six studies assessed secondhand exposure to vaping product aerosol, using a variety of biomarkers, none from the UK. The level of exposure varied greatly from people at home to people attending an indoor vaping convention.

Short exposures to secondhand vaping did not result in detectable changes in levels of nicotine, VOCs or TSNAs. However, longer exposures during heavy sustained vaping were associated with significant increases in nicotine or potential toxicants' metabolites.

Implications

Our systematic review covered a wide range of biomarkers and studies. Our findings are broadly consistent with the few previous reviews in this area, but because of the greater volume of research that has been conducted in recent years, the implications are much clearer.

Vaping reduces toxicant exposure compared with cigarette smoking

The reviewed studies show that compared to smoking, using vaping products leads to a substantial reduction in biomarkers of toxicant exposure associated with cigarette smoking. However, the degree of any residual risk remains unclear, mainly because of the lack of comparisons between long-term former smokers who do and do not vape or comparisons with those who have never smoked or vaped.

Methodological improvements needed

Our quality assessments revealed most studies had some methodological concerns, and these should be addressed in future research as they limit interpretations of our findings. For example, a lack of significant differences between levels of exposure between people who vape and non-users may be due to several reasons. This includes a lack of sensitivity in biomarker measurement methods, background environmental exposures, or because exposure to potential toxicants between people who only vape and non-users is relatively similar.

Improvements in definitions needed

Historical tobacco use can greatly affect many of the biomarkers used to determine exposure to potentially harmful constituents from vaping. So, as most vapers are previous long-term smokers (see chapter 4 on vaping among adults), strict definitions for duration of exclusive vaping (only vaping) should be used consistently in future studies.

Similarly, definitions should not include concurrent smoking, and only include people who exclusively vape. This is particularly important for cross-sectional studies, but longitudinal studies should also use objective measurements to assess concurrent cigarette smoking.

Future studies should always verify biologically participants' smoking, vaping or non-use status, rather than rely on self-reports. Based on our review findings, measurements of carbon monoxide or NNAL could be used to improve over-reliance on self-reported vaping and smoking.

More research needed on biomarkers of exposure among vapers

More research is needed on biomarkers of exposure among vapers, particularly in the UK, where we identified a lack of studies. We would encourage research with longitudinal and cross-sectional designs. While longitudinal research is more robust, particularly in relation to changes over time, cross-sectional research also offers insight into exposure from realistic and naturalistic use patterns. Longitudinal research would benefit from including longer follow-up periods to be able to assess long-term changes in biomarker exposure among vapers who sustain use over long periods of time (see chapter 4 on vaping among adults). This is also important for biomarkers with longer half-lives.

In our meta-analyses, many findings were from tobacco industry-funded RCTs conducted in confinement (closed settings such as research centres or hospitals in which the participants stay) for periods of up to 7 days. So, future research needs to include more independent research of biomarkers of exposure in people who use vaping products, smoke and do not use tobacco or nicotine outside of confinement (in their own normal settings), and with longer follow-ups.

Need to distinguish between biomarkers of exposure from tobacco and from other sources

Several biomarkers of exposure are not specific to tobacco, and almost all biomarkers are susceptible to the effects of confounders (which can also influence levels of a biomarker). For example, VOCs are prevalent in many household products such as paints and cosmetics and can also be influenced by diet.

Where a person lives can also uniquely influence exposure. There are higher levels of polyaromatic hydrocarbons and other toxicants found in urban environments due to motor vehicle exhaust fumes and other sources of pollution. There are also different toxicant exposures in rural environments, due to pesticide exposure and other agricultural pollutants.

So, strict control for confounders and large sample sizes are needed to reduce the influences of other environmental exposure on findings in cross-sectional research.

Need to identify and study biomarkers which are specific to vaping

Our systematic review used the World Health Organization (WHO) priority toxic contents and emissions list for tobacco products. There are already suggestions to include vaping specific biomarkers in the WHO list when and if these emerge, which will help guide future research. Due to the variety of different metal elements used for vaping product components, there may be exposure to certain metals from vaping that are not present in exposure from tobacco. Future research is needed to identify types of metal exposure that are exclusively from vaping products and how these can be mitigated.

There is a need to address the lack of comparable research on biomarkers of exposure to nicotine and potential toxicants across different groups, such as:

- young people and adults
- different genders
- ethnicity
- socioeconomic status

Given we identified no studies assessing the biomarkers of exposure to vaping among people with existing health conditions on disease outcomes, this is an important gap that should be addressed by funding bodies.

Lower risks of exposure from vaping than smoking

Overall, despite the methodological limitations identified in our systematic review, evidence suggests significantly lower relative exposure from vaping compared to smoking in biomarkers that are associated with the risk of:

- cancer
- respiratory conditions
- cardiovascular conditions
- other health conditions

This is consistent with encouraging people who smoke to switch completely to vaping as a way to stop smoking or as alternative nicotine delivery devices. Also, our findings of higher absolute exposure from vaping compared with not using any nicotine products reinforce the need to discourage people who have never smoked from taking up vaping (or smoking).

Chapter 8: Biomarkers of potential harm to health cutting across several diseases

Evidence reviewed

This chapter examines findings from our systematic review on biomarkers of potential harm to health that are associated with:

- oxidative stress
- inflammation
- endothelial function
- platelet activation

These biomarkers are known to be associated with the development of multiple diseases (see chapter 2, table 6). So, they are relevant to both our review questions:

1. What effect does vaping have on biomarkers that are associated with the risk of cancer, respiratory, cardiovascular and other health conditions?

2. What effect does vaping among people with existing health conditions have on disease outcomes?

Several of the studies we included assessed biomarker changes in participants with existing health conditions (for example, asthma and dental diseases) but did not estimate how these changes affected outcomes of these health conditions. As these studies did not directly address the second review question, we have presented their data alongside findings from participants from the general population.

Main findings

Issues caused by differences between studies

Overall, we identified 41 unique studies in 43 publications that reported biomarkers of potential harm associated with oxidative stress, inflammation, endothelial function and platelet activation biomarkers. There were significant differences in methodologies across the studies we included, which likely resulted in discrepancies and variability of findings. These differences included the following.

1. Studies assessed multiple biomarkers with different sensitivity, speed of onset or offset and reliability of predicting subsequent health risks. These differences obscured overall conclusions.

2. Studies used different definitions for vaping, smoking and non-use groups, usually did not bioverify smoking or vaping status, and used varied methods (for example, different measures, biosamples and follow-up times) to compare a range of biomarkers between these groups. These differences prevented us pooling data from more studies for meta-analyses and made comparisons between studies complicated.

3. Most studies we included assessed acute vaping effects on oxidative stress, inflammation, endothelial and platelet functions. And because the explored biomarkers of potential harm mostly take weeks or months to normalise after people stop smoking, we cannot make clear conclusions about longer-term vaping effects.

4. Tobacco smoking (or vaping) is not the only known risk factor for detrimental changes in many of the explored biomarkers. And conclusions about vaping associations with the explored biomarkers are further limited by potential confounding of other variables and the lack of controlled studies. So, findings need to be cautiously interpreted.

In line with our algorithm (chapter 2, table 6), we carried out meta-analyses wherever possible, but a lack of consistency in study designs, biomarker reporting, group definitions and exposure periods resulted in few studies being included.

Oxidative stress

One RCT, 6 cross-over studies, 5 non-randomised longitudinal studies and 11 cross-sectional studies assessed oxidative stress biomarkers, specifically:

- low-density lipoprotein (LDL)
- high-density lipoprotein (HDL)
- 8-isoprostane
- soluble Nox2-derived peptide (sNOX2-dp)
- malondialdehyde (MDA)
- 8-hydroxy-2'-deoxyguanosine (8OhdG)
- reactive oxygen species (ROS)

We found no significant differences in LDL levels across studies between vapers, smokers and non-users' groups after acute and short-to-medium exposure. A meta-analysis of data from 2 cross-sectional studies also confirmed no difference in blood LDL levels between vapers and non-users.

Findings on HDL levels were inconsistent. Smaller studies reported no differences between vapers, smokers and non-users, and larger studies reported lower HDL levels among non-users compared with vapers and smokers. Two meta-analyses of cross-sectional studies found no difference in blood HDL levels between vapers compared with smokers or non-users.

Evidence for 8-isoprostane level changes after vaping product use was mixed. Studies emphasised longer past smoking history, older age and female gender as potential confounders for higher 8-isoprostane levels (these factors are associated with higher 8-isoprostane levels). In general, comparisons were limited by a lack of longer-term controlled exposure studies (considering time for biomarkers' levels to normalise) and potential confounding in non-randomised longitudinal and cross-sectional studies.

There was limited evidence for other oxidative stress biomarkers. The overall evidence from most of the included studies show no difference in vaping-associated oxidative stress risks in comparison with smoking or not using tobacco or nicotine products.

Inflammation

Two RCTs, 3 cross-over studies, 3 non-randomised longitudinal studies and 17 cross-sectional studies assessed inflammation biomarkers, specifically:

- white blood cell (WBC) count
- c-reactive protein (CRP)
- interleukin-6 (IL-6)
- interleukin-8 (IL-8)
- tumour necrosis factor alpha (TNF-α)
- soluble intercellular adhesion molecule 1 (sICAM-1)
- fibrinogen
- prostaglandin E2 metabolite (PGE-M)
- monocyte chemoattractant protein-1 (MCP)

Pooled data from 3 cross-sectional studies showed that average blood CRP levels were lower among vapers than smokers and similar between vapers and non-users, and that average blood sICAM-1 levels were significantly lower among vapers than smokers. However, controlled and longitudinal studies did not confirm these cross-sectional findings. Also, due to varied study designs and a lack of studies comparing the same outcome between the same study groups, no definite conclusions could be drawn on the association between vaping and any specific inflammation biomarker.

Endothelial function

One RCT, 4 cross-over studies, 3 non-randomised longitudinal studies, and one cross-sectional study assessed endothelial function biomarkers, specifically:

- flow-mediated dilation (FMD)
- E-selectin and P-selectin
- nitric oxide
- microvesicles

No studies reporting on these biomarkers could be pooled for a meta-analysis.

While acute exposure studies showed similar short-term reductions in FMD parameters after vaping (with and without nicotine) and smoking sessions, a single RCT showed that switching from smoking to vaping for 4 weeks significantly improved (increased) participants' FMD function.

Evidence from 2 cross-over studies and one interventional study showed that acute vaping and smoking sessions led to similar reductions in nitric oxide bioavailability (more susceptibility to oxidative damage), but one study also noted that the reduction was directly associated with the length of past smoking history.

Evidence from one cross-over study and one interventional study showed that acute nicotine vaping increased blood endothelial microvesicle levels while acute non-nicotine vaping did not change this outcome.

There was limited and inconsistent evidence on the other endothelial function biomarkers. Also, we could not make any conclusions about the absolute effect of vaping on endothelial function, as no controlled studies compared vapers and non-users.

Overall, acute vaping might cause endothelial dysfunction as much as acute smoking but switching from smoking to vaping product use might improve endothelial function in the longer-term.

Platelet biomarkers

Only one cross-over study, one longitudinal study and 2 cross-sectional studies assessed platelet activation measures. No data from these studies could be meta-analysed. So, evidence on the association between vaping and platelet function was limited, and we could not make any conclusions about absolute effects of vaping on platelet activation or effects of vaping relative to smoking.

Implications

Need for methodological improvements and longer term studies

Considering the 2 human studies summarised by the NASEM report and the 41 studies (in 43 publications) included in our systematic review, research on effects that human vaping has on biomarkers that cut across diseases has grown in recent years, though it is still at an early stage.

Our summary of the evidence on associations between vaping and oxidative stress, inflammation, endothelial function and platelet activation came from studies with different methodologies that mostly assessed acute exposure effects. These findings provide important insights allowing us to compare immediate effects between vaping and smoking. However, like smoking, it is the effects of long-term vaping that will be most relevant to

public health, and the explored biomarkers of potential harm mostly take weeks or months to normalise after people stop smoking.

Our risk of bias assessments showed that most studies in this chapter had methodological concerns, and these should be addressed in future research as they limit interpretations of our findings. More research is needed, particularly in the UK, where we identified a lack of studies.

There is a need for future research among people who vape and have never smoked. This would allow us to determine long term changes in biomarkers of potential harm exclusively due to vaping and not as a consequence of prior long-term smoking.

Need to distinguish between biomarkers of potential harm from smoking or vaping from other sources

Also, most biomarkers of potential harm are associated with multiple confounders not related with vaping or smoking (for example diet, physical activity). So, studies that explore acute effects of vaping and/or smoking, but do not include non-users as a comparison group, cannot clearly distinguish between the effects of vaping and/or smoking on these biomarkers. Due to these reasons, most studies that we have summarised in this chapter cannot inform us about the medium or long term vaping-associated risks via effects on the biomarkers we reviewed. This implies that further controlled studies with adequate sample sizes, non-user comparison groups, and longer exposure and follow-up times are needed to clarify how switching from smoking to vaping affects the most reliable biomarkers of harm.

Greater clarity on clinical significance

More research is also needed to develop ranges where biomarkers of potential harm become clinically relevant predictors of disease. This would improve the biomarkers' ability to estimate the pathways and contributions of vaping and smoking to multiple diseases.

Chapter 9: Cancers

Evidence reviewed

In this chapter we reviewed the existing evidence on how vaping might affect cancer risk. This included summarising previous reports that have addressed this issue, and then presenting findings from our systematic review of health risks and effects of vaping that are relevant to cancer.

Main findings

Toxicants and carcinogens

Our 2018 evidence review of vaping, the report from NASEM and the COT report include some earlier evidence. The 2018 report included one study directly relevant to cancer that suggested people who switched from smoking to vaping were exposed to lower levels of toxicants and carcinogens than in smoking, but also pointed to the need for further research. The NASEM report found no available evidence about whether the chemicals in vaping aerosols or vaping behaviour were associated with cancer risk relative to smoking or non-use. COT also reported that existing evidence was insufficient to draw conclusions about any links between vaping and cancer risk in humans.

Cancer risks

We identified a growing (but still modest) amount of literature on how vaping may affect cancer risks in humans. In our review of human studies, biomarkers of exposure to several human carcinogens in tobacco smoke show lower measured levels in people who vape compared with those who smoke. So, the biomarker of exposure studies compiled in this review (see chapter 7 on biomarkers of exposure) provide conclusive evidence that vaping generally leads to lower exposure to many of the carcinogens responsible for the health risks of smoking.

Inflammation and oxidative stress

Findings from studies of inflammation and oxidative stress do not show any systematic relationship with mixed evidence of differences (or no difference) in levels between vapers and smokers and non-users. So, this evidence is currently insufficient to draw conclusions.

Gene and DNA processes

We identified 2 RCTs, one longitudinal study and 5 cross-sectional studies of gene expression and DNA methylation in humans (none from the UK). Methodological limitations (for example, a lack of smoking comparison groups in some studies) constrain what we can say about these epigenetic studies (the study of how people's behaviours and environment can cause changes that affect the way our genes work). Even so, methylation and demethylation of specific genes related to smoking and vaping show potential for achieving more clarity in this area.

Existing or previous cancer conditions

There were no studies that assessed how vaping affects people with an existing or previous cancer condition.

Cell and animal studies

It is challenging to interpret the findings from pre-clinical studies using human or animal cells or rodent models to any cancer risks arising from vaping in humans. These pre-clinical studies commonly use acute exposures, sometimes over concentrated periods. So, it is unclear whether the pathways to risk identified would be replicated in vapers. Further challenges arise because of the complex nature of vaping behaviour over time and the wide variety of different aerosols and products used.

Despite these significant limitations, there are suggestions from this literature that vaping aerosols are not benign to people who have never smoked. And that exposure to these aerosols may be implicated in negative outcomes that could affect the viability of cancer treatment for people with pre-existing disease. However, cell and animal studies appear to support the human studies and suggest vaping may trigger alterations in gene expression, but at a lower extent than we see from exposure to tobacco smoke.

Implications

Longer follow up periods are needed

Vaping generally leads to lower exposure to many of the carcinogens responsible for the considerable health risks of smoking. However, studies of biomarkers of exposure that are associated with cancer risk in humans need to have longer follow up periods than has been the case to date, as this will give us better information if vaping reduces cancer risk compared with smoking.

More research is needed

More research is needed on biomarkers of potential harm in humans.

Studies applying potentially important new methods to assess vaping often neglect to include cigarette smoke as a comparator as well as a control (usually filtered air). Even when a tobacco smoke comparison group is included, it is often difficult to compare like with like when the exposure to nicotine and other important parameters are not included in the description of the experiments. Such data are essential when assessing whether human exposure to different forms of nicotine delivery (in this case vaping and smoking) result in different magnitudes of cancer risk.

Further studies are needed to identify the extent to which evidence from pre-clinical studies is directly relevant in humans.

There are a number of gaps in the literature identified in our review, as well as some gaps that came to our attention when preparing the background to this chapter. Although we know a lot about the links between tobacco smoking and cancer, more needs to be done

to document the smoking status of cancer survivors. These people will make up an increasing proportion of cancer patients in the future given improvements in survival and an ageing population. This means that the risk of recurrence or a secondary cancer will not be uncommon.

We could also not identify any studies from the UK on vaping prevalence among people diagnosed with cancer or cancer survivors, so this should be a further area of research.

More research is needed with cancer patients and cancer survivors to understand any role for vaping as a smoking cessation aid in improving treatment outcomes or reducing the risk of cancer recurrence.

Studies are also needed that assess the effects of vaping on cancer outcomes in people diagnosed with cancer, both to compare with people not using nicotine or tobacco products and with people smoking.

Support smokers to completely switch from smoking to vaping

For policy makers and practitioners, findings from our review for this chapter suggest that developing and implementing policies and interventions that support smokers to completely switch from smoking to vaping will reduce exposure to toxicants and carcinogens. This may have relevant outcomes for cancer prevention.

Chapter 10: Respiratory diseases

Evidence reviewed

In this chapter we reviewed the existing evidence on how vaping might cause or influence respiratory disease, one of the main causes of premature mortality and morbidity among smokers. This included summarising previous reports that have addressed this issue, and then presenting findings from our systematic review of health risks and effects of vaping that are relevant to respiratory disease. Our systematic review aimed to assess the effects of exposure to vaping on biomarkers associated with the risk of poor health conditions and to assess the effect of vaping on disease outcomes in people with existing health conditions.

Most studies examined healthy participants, which we summarise first. We then summarise the studies that examined participants with respiratory conditions (asthma, chronic obstructive pulmonary disease) and smokers with mental health conditions. We assessed both relative and absolute vaping risks associated with biomarkers of respiratory disease where the data were available (between vapers and smokers, and between vapers and non-users), and where feasible included comparisons across different population groups.

Conclusions for biomarkers of exposure and biomarkers of potential harm cutting across common diseases are presented in chapters 7 and 8.

Main findings

Biomarkers of respiratory diseases

Several biomarkers of exposure are relevant to respiratory diseases. We identified conclusive evidence that under typical use conditions, acute (from single use to 7 days) and short to medium (from 8 days to 12 months) exposure to most potential respiratory toxicants from vaping is significantly lower compared with smoking tobacco cigarettes. And there are substantial reductions in some biomarkers. For the respiratory toxicants that were assessed at long-term exposure (more than 12 months), evidence was moderate that biomarkers of exposure are lower for vaping than smoking.

For a few VOCs, such as formaldehyde and toluene, available evidence was inconclusive on the significant differences between vapers and smokers. However, one study suggested formaldehyde exposure might increase during compensatory puffing behaviour with lower nicotine strength e-liquids. In general, there were no significant differences between vapers and non-users, except for acrylonitrile metabolite CNEMA. The evidence suggested that vaping might increase exposure to acrylonitrile in absolute terms.

Biomarkers of potential harm

The evidence was mixed on biomarkers of potential harm relevant to multiple diseases (including respiratory disease), such as 8-isoprostane and inflammation. This would indicate that there was insufficient evidence from these biomarkers of potential harm whether vaping product use is associated with respiratory disease in humans.

We identified 25 studies (3 from the UK) that assessed other biomarkers of potential harm that were specifically related to respiratory disease in humans. Consistent with studies in other chapters, the studies we included used a range of different designs and had varying quality or risk of bias. Studies used a range of different definitions of vaping and smoking. For example, findings of some studies were confounded by categorising vapers who smoke, occasional vapers or exclusive daily vapers as a uniform group or comparing occasional vapers with daily smokers. So, findings need to be cautiously interpreted.

Studies with more than one time point mostly explored acute exposure to vaping or followed-up participants for short to medium term. In line with our algorithm for selecting studies for meta-analyses (chapter 2, table 6), the lack of consistency in study designs, biomarker reporting, group definitions and exposure periods meant we were unable to carry out any meta-analyses.

Of the 25 studies we included:

- 7 were relevant to our second research question about effects of vaping among people with existing health outcomes on disease outcomes
- 4 assessed participants with asthma
- 2 were from the same longitudinal cohort, but with different follow-up rates, assessing participants with chronic obstructive pulmonary disease (COPD)
- 1 assessed participants with mental health disorders

Respiratory tests and imaging

All 25 studies included spirometry measures, which is a breath test used to assess airflow obstruction in the lungs (commonly used to detect respiratory diseases). But the different study designs, groups and duration of exposure limited any conclusions that we can draw.

Overall, the findings showed no acute (from single use to 7 days), short to medium (from 8 days to 12 months) or long-term (more than 12 months) detrimental effects for vapers. Whereas a clear worsening of lung function was seen in one small study of vapers who switched back to smoking for 7 days.

Eight studies assessed FeNO (fractional exhaled nitric oxide, which is measured in the breath and is a marker of airway inflammation and asthma). Again, these studies involved different designs, groups and exposure duration so limited our conclusions. There were mixed findings in the studies, but most reported no significant differences across the user groups.

One study assessed impulse oscillometry (a respiratory diagnostic test), which suggested an effect of acute nicotine exposure on some lung function attributes among healthy occasional smokers but needs repeating.

Five imaging and bronchoscopy studies used a variety of different techniques. These studies either assessed very short-term single-use exposure or were heavily confounded by including smokers (either of tobacco or marijuana) in the vaping groups.

Overall, given the methodological differences, we concluded that there was insufficient evidence from spirometry, FeNO, impulse oscillometer, and bronchoscopy and imaging studies as to whether vaping has any impact on lung function after acute, short to medium and long-term exposure. We also concluded that there was insufficient evidence on whether acute secondhand vaping had any effect on lung function.

Asthma

In relation to our second research question, we first summarise our findings from the 4 studies with participants with asthma. Again, sample sizes were generally very small, and the findings were inconclusive as to whether there are improvements in lung function and respiratory symptoms among adult smokers with asthma who switch to vaping completely.

There was limited evidence that vaping negatively affects lung function among adults with asthma.

Chronic obstructive pulmonary disease

Two longitudinal articles taken from the same group of COPD patients reported that they found statistically significant improvements in some spirometry measurements for the group who used vaping products compared with baseline. But there were no significant differences in the group who smoked. However, only small numbers of participants were involved, and the authors suggested larger studies were needed to confirm these findings.

These findings indicate that there is limited evidence for reduction of COPD exacerbations among adult smokers with COPD who switch to vaping completely and continue vaping for up to 5 years.

Mental health

In one study, smokers with a mental health diagnosis were encouraged to use a vaping product to reduce smoking, and reported no statistically significant changes in one spirometry measure. But since most of them continued smoking, further research is needed with this population.

Cell and animal studies

As previously mentioned, it is challenging to directly translate the findings from pre-clinical studies using human or animal cells or rodent models to any respiratory risks arising from vaping in humans. These pre-clinical studies commonly use acute exposures sometimes over concentrated periods, and it is unclear whether the mechanisms or pathways to risk identified would be replicated in vapers. Further challenges arise because of the complex nature of vaping behaviour over time and the wide variety of different aerosols and products used.

We identified 47 in vitro studies that examined biological impact of exposure to vaping product aerosol or vaping product aerosol extract on various human airway cell types. We also identified 25 animal studies investigating respiratory effects following vaping product exposure.

Taking all the reviewed articles into consideration, the current available data contributes to the evidence that vaping product aerosol, to some extent, may cause airway-related adverse effects in cell and animal models. Although, the evidence is inconclusive as to which constituents of the aerosol play important roles in the observed cellular and physiological effects.

Conclusions

Overall, while the literature has grown considerably since the NASEM report, the conclusions from that report are supported by this review.

The lack of consistency across the studies meant we could not perform meta-analyses of respiratory measures, which limits the conclusions that we can draw.

The limited evidence for improvements in COPD for adult smokers in the NASEM report who switched to vaping has now been reported at the 5 year follow-up by the same study group. This shows that improvements seem mainly to be among participants who switched to exclusive vaping.

More studies have been carried out with people suffering from asthma, but the different designs, diagnoses, and measurements taken prevent us from making any conclusions.

Implications

Improve research methodology

Our quality assessments revealed most studies had some methodological concerns, and these should be addressed in future research as they limit interpretations of our findings. More research is needed, particularly in the UK, where we identified a lack of studies.

As we previously mentioned, all studies we included had used very different methods. This included different designs, definitions of user groups (people who smoke, people who vape, people who smoke and vape, and people who do neither) and biomarkers. This likely resulted in discrepancies and variability in their findings.

Study people who vape over longer periods of time

As discussed in other chapters, most studies exposed participants to brief sessions of vaping, so cannot answer questions on long-term respiratory outcomes. So, studies that assess people who have been vaping over long periods of time are urgently needed. Findings from one long-term group of smokers who had switched to vaping at baseline are promising and should be replicated by other studies with larger numbers of participants.

More studies are needed that compare long-term former smokers who do and do not vape, as well as studies comparing former smokers who vape with people who vape who have never smoked.

Research on vaping needs to measure strength of evidence

As many studies involve small numbers of participants, researchers should use other, less traditional ways to test their findings. This could include using a Bayes factor analysis to measure the strength of evidence. This is relevant to findings from most of the health biomarker studies included in this report.

Switching to vaping likely to slow down respiratory disease development

For policy makers and practitioners, the limited evidence from our review for this chapter suggests that developing and implementing policies and interventions that support smokers to completely stop and switch to vaping is likely to slow down the development of respiratory diseases.

Consider respiratory biomarkers before starting studies

Researchers need to carefully consider their choice of respiratory biomarkers before carrying out their studies. While some found statistically significant changes in spirometry measures, it is not clear whether these changes are too small to be clinically relevant. This raises the question of how useful spirometry measures are in detecting any vaping risks, particularly among healthy smokers. This concern also applies to other biomarkers, such as inflammatory changes. Also, the pathways between these biomarkers and an increased risk of certain respiratory diseases still needs to be clearly mapped out with supportive evidence.

Considerations for human cell studies

For human cell studies, biologically relevant doses of nicotine or flavours that mimic exposure to vaping product aerosol emissions are needed.

Seek a global consensus on measuring changes to the respiratory system

Studying changes to the respiratory system is important as these might be the first signals of potential harms or (relative) benefits from vaping. So, seeking a global consensus on what measures should be studied, as well as over what duration of exposure and follow-up, is urgently needed.

Assess effects of vaping on people with existing respiratory problems

More studies are needed that assess the effects of vaping on people with pre-existing respiratory problems or diseases. This includes both in comparison with no use of nicotine or tobacco and in comparison with smoking.

Chapter 11: Cardiovascular diseases

Evidence reviewed

For cardiovascular diseases, we did not identify any studies on people with existing cardiovascular conditions so we could not address the second aim of the review. We assessed both relative and absolute vaping risks associated with biomarkers of cardiovascular disease where the data were available (between vapers and smokers, and between vapers and non-users). And where feasible, we included comparisons across different population groups.

We present our conclusions for biomarkers of exposure and biomarkers of potential harm across several diseases in chapters 7 and 8.

The studies we reviewed show that compared to smoking, using vaping products leads to a substantial reduction in biomarkers of toxicant exposure. However, the degree of any residual risk (from vaping but also previous smoking and other factors affecting cardiovascular health) remains unclear, mainly because of the lack of studies using appropriate comparators.

Main findings

Cholesterol

Looking at biomarkers of potential harm relevant to multiple diseases, studies of low-density lipoprotein (LDL) cholesterol showed no differences after acute and short-to-medium use of vaping products, smoking or non-use. LDL cholesterol is sometimes described as ‘bad cholesterol’ as it makes heart problems or a stroke more likely. Similar findings were seen for high-density lipoprotein (HDL) cholesterol (or ‘good cholesterol’), except among large-scale samples of non-users where HDL levels were significantly higher than among vapers and smokers.

Oxidative stress

The findings were more mixed for markers of oxidative stress 8-isoprostane and sNOX2-dp. However, as these oxidative stress biomarkers are influenced by other factors, we could not make strong conclusions on their associations with vaping product use.

Inflammation

For inflammation markers, differing study designs prevented us from making strong conclusions. The meta-analyses of cross-sectional studies suggested lower levels of the inflammation biomarkers (blood CRP and sICAM-1) among vapers than smokers, and similar levels between vapers and non-users. But these findings were not confirmed by other interventional studies that largely focused on acute and short-term exposure.

Endothelial function

For endothelial function biomarkers, a single RCT found that switching from smoking to vaping improved FMD after one month. Evidence from the other studies suggested a short-term deterioration in FMD after acute exposure to vaping product use. Evidence from the other endothelial function biomarkers and the 4 studies on platelet activation markers was also difficult to synthesise. This was due to different designs, outcome measures and comparison groups.

Harm specific to cardiovascular disease

We identified 41 studies that assessed biomarkers of potential harm specific to cardiovascular disease in humans. Consistent with studies in other chapters, the studies we included:

- used a range of different designs
- had varying quality or risk of bias
- used a range of different definitions of vaping and smoking

Studies with more than one time point mostly explored acute exposure to vaping or followed-up participants for short to medium term. So, we were unable to summarise findings on longer-term vaping exposure. In line with our algorithm (chapter 2, table 6), we carried out meta-analyses wherever possible, but a lack of consistency in study designs, outcome reporting, group definitions and exposure periods resulted in data from few studies being meta-analysed.

Heart rate

Thirty-one studies assessed heart rate in humans (4 studies from the UK), and 9 of them could be included in meta-analyses. We were able to conduct 2 meta-analyses of findings comparing vaping and smoking (3 cross-over and 2 cross-sectional studies), 2 meta-analyses of findings comparing vaping and non-use (3 cross-over, 2 cross-sectional studies) and one meta-analysis of findings comparing vaping and non-nicotine vaping (4 cross-over studies).

Acutely, immediately after use, vaping increased heart rate less than smoking. Heart rate after short exposure to vaping was similar to heart rate after not using tobacco or nicotine products. There was no difference in heart rate after nicotine and non-nicotine vaping. Any differences may vary with devices, liquids and puffing behaviours influencing the amount of nicotine delivered and this is further explored in chapter 5 on nicotine.

Comparing longer-term changes in heart rate, people who vaped had lower heart rate than people who smoked when the groups were mutually exclusive (people who vaped did not also smoke). Compared with people who did not vape or smoke, heart rate among people who vaped was lower in a meta-analysis of cross-sectional studies but higher in another cross-sectional study. One longer-term study reported the same level of change in heart rate for smokers who started using nicotine or non-nicotine vaping products.

Blood pressure

Thirty studies assessed blood pressure in humans (3 studies from the UK), with 9 studies that could be included in meta-analyses. We conducted 4 meta-analyses of findings comparing blood pressure when vaping and smoking (3 cross-over studies, 2 cross-sectional studies, meta-analysis repeated for systolic (when your heart beats) and diastolic (when your heart rests between beats) blood pressure), 4 meta-analyses of findings comparing vaping and non-use (3 cross-over and 2 cross-sectional studies, again for both systolic and diastolic blood pressure) and 2 meta-analysis comparing nicotine and non-nicotine vaping (4 cross-over studies, again for both systolic and diastolic blood pressure).

Meta-analyses comparing acute effects found no differences in blood pressure after vaping, smoking or doing neither with the exception of a small difference between vaping and non-use for diastolic blood pressure. Studies that could not be meta-analysed found mixed results. A meta-analysis comparing acute effects of nicotine and non-nicotine vaping found no difference as did most other studies that could not be meta-analysed but included non-nicotine vaping.

Meta-analyses of cross-sectional studies where participants had had longer exposure to vaping (at least 3 months or one year) found that people who vaped (presumably mostly former smokers) had lower blood pressure than people who smoked. There was no difference between people who vaped and people who did not vape or smoke. Studies that could not be meta-analysed found mixed results regarding change in blood pressure.

Secondhand exposure

Only 2 small studies at serious risk of bias included secondhand exposure. So, we could not draw conclusions about what effects exposure to secondhand vapour has on heart rate or blood pressure.

Peripheral resistance and arterial stiffness

Nine studies assessed peripheral resistance or arterial stiffness (PWV) in humans (one study from the UK). Results could not be meta-analysed. PWV may decrease (improve) after smokers have switched to vaping for a sustained period. However, the longest follow-up reported was only 4 months.

PWV generally increased after acute exposure to vaping nicotine, but not after non-nicotine vaping, suggesting that any acute effects of vaping on PWV are due to nicotine.

Oxygen saturation

Three studies (all at critical risk of bias, none from the UK) assessed acute effects on oxygen saturation in humans. Results could not be meta-analysed, and we could not draw conclusions based on the available evidence.

Cell and animal studies

Evidence from cell studies was very limited, with only 2 studies identified in our review. Results showed that vaping product aerosol increased damage to cells and that effects varied across different flavours.

Sixteen studies in animals were included. In summary, animal studies showed that exposure to vaping product aerosol increases blood pressure. Some studies found a decrease in heart rate, although most found no effect.

Animal studies also show an increase in biomarkers of arterial stiffness linked to exposure to vaping products. This may be similar to or smaller than increases caused by smoking. Left ventricular mass and vessel wall thickness (in the heart) were increased and left ventricular function reduced after vaping product aerosol exposure. These effects were potentially less than for exposure to cigarette smoke, and there were inconsistencies in findings across studies. These vaping product-induced effects appeared largely to be nicotine-dependent.

Exposure to vaping product aerosol was associated with decreases in animals' blood vessel health, as well as increases in markers of thrombosis risk, inflammation, oxidative stress, scarring, and cell health. Although, it is inconclusive as to which constituents of the aerosol play important roles in the observed effects.

As previously mentioned, it is challenging to directly translate the findings from pre-clinical studies using human or animal cells or rodent models to any cardiovascular risks arising from vaping in humans. These pre-clinical studies commonly employ acute exposures, sometimes over concentrated periods, and it is unclear whether the mechanisms or pathways to risk identified would be replicated in people who vape.

Role of nicotine

The evidence does not allow us to distinguish pathways to cardiovascular disease. One potential pathway is through nicotine, and the biomarkers of exposure and pharmacokinetic studies show that people who vape can achieve nicotine levels similar to people who smoke. The animal studies suggested that nicotine did play a role in some of the changes seen in cardiovascular biomarkers, specifically:

- blood pressure
- arterial stiffness
- left ventricular mass and function

Some studies included in this chapter assessed cardiovascular biomarkers in humans through non-nicotine vaping as well as nicotine vaping. This could help explain the assumed role of nicotine in any cardiovascular risks of vaping for humans. However, the differences between studies limits our conclusions.

Meta-analyses of cross-over studies from vaping nicotine and non-nicotine products for heart rate and blood pressure found no differences. Studies that we could not meta-analyse did not consistently find this. The findings were more consistent in PWV effects where nicotine did appear to be implicated at least in acute studies.

Comparisons with other reports

Conclusions from the NASEM report are generally supported by this review. As in 2018, to date there is still no available evidence on whether vaping is associated with clinical cardiovascular outcomes (coronary heart disease, stroke, and peripheral arterial disease) and subclinical atherosclerosis (carotid intima-media thickness and coronary artery calcification).

The NASEM report found substantial evidence that heart rate increased shortly after nicotine intake from vaping, which was also seen in this review (whereas evidence was inconsistent for non-nicotine vaping).

NASEM found moderate evidence that diastolic blood pressure increases shortly after nicotine intake from vaping and limited evidence that vaping is associated with a short-term increase in systolic blood pressure. Based on the still limited and mixed evidence, we conclude that there may be reductions in blood pressure after people who smoke switch to vaping and little difference between people who vape and people who do not vape or smoke.

The NASEM report also concluded that there was insufficient evidence that vaping was associated with long-term changes in heart rate, blood pressure, and cardiac geometry

and function. In our review, evidence from animal studies suggests that there may be some long-term changes, but we found no evidence from human studies. And, as already discussed, the validity of animal studies for human outcomes has limitations.

Similarly, conclusions by COT are generally supported by this review. COT concluded that exposure to nicotine from vaping was unlikely to be higher than from smoking. This is confirmed by studies included in this review that found no significant difference between people who vaped or smoked at least weekly.

COT also concluded that vaping was associated with some emissions into ambient air, including nicotine, so that pharmacological effects from exposure to nicotine in ambient air may occur in some individuals. In this review, only 2 small studies at serious risk of bias assessed short-term secondhand exposure to nicotine vaping. So, this did not allow us to make any clear conclusions.

Conclusion

Overall, the extent to which vaping presents a risk for cardiovascular health remains uncertain. But based on the toxicant profile in vaping products and aerosols, the risk is expected to be much less than that of cigarette smoking.

Implications

Our quality assessments revealed most studies had some methodological concerns, and these should be addressed in future research as they limit interpretations of our findings. More research is needed, particularly in the UK, where we identified a lack of studies.

Most studies exposed participants to brief sessions of vaping (27 out of 41 included studies were cross-over or acute exposure studies). And although it can address questions about immediate effects of vaping, this study design is not able to answer questions about effects on the cardiovascular health outcomes most relevant to public health.

Studies that compare rates of cardiovascular diseases between non-users, users of tobacco and users of nicotine vaping products are needed (for example, rates of coronary heart disease, peripheral arterial disease and stroke).

Studies should include longer-term follow-ups and more informative measurements. Studies measuring heart rate or blood pressure should try to include 24 hour ambulatory blood pressure and heart rate. This would improve the validity of the measurement rather than rely solely on measurements in single or short sessions. Researchers should consider including heart rate variability (a higher variability can indicate better health) as an outcome measure, for example in people who switch from smoking to vaping. Evidence is also needed on the extent of longer-term changes in other outcomes such as PWV.

Alongside longer follow-ups, inclusion of long-term exclusive vapers may also help address this.

Historical tobacco use can greatly affect many of the biomarkers used to determine exposure to potentially harmful constituents from vaping. As most vapers are previous long-term smokers (see chapter 4 on vaping among adults), definitions for vaping should preclude concurrent smoking and a minimum duration of exclusive vaping should be defined. Studies are needed that compare long-term former smokers who do and do not vape, as well as studies comparing former smokers who vape with people who vape who have never smoked.

Compliance with study allocation and definitions of groups should be verified and reported in all studies. For example, the level of CO exhaled by people categorised as not smoking and the level of nicotine in people categorised as vaping or not using any nicotine products.

The existing evidence does not provide insights into the effects of vaping on cardiovascular health in people of different sex, age or ethnicity. So, future research should pay attention to groups with different cardiovascular risk profiles.

Studies are needed that assess the effects of vaping on people with pre-existing cardiovascular conditions, both in comparison with not using nicotine or tobacco and in comparison with smoking.

Cardiovascular health and disease are affected by a wide range of genetic predispositions, behavioural risk factors and environmental exposures. Further research is needed to clarify any unique contributions from vaping while accounting for other factors.

Vaping products vary and any effects on cardiovascular health are likely to differ with device types, nicotine concentration, liquid composition and user behaviours. As one example, most studies in the US used nicotine concentrations above the legal threshold in the UK and EU, but we were unable to run meta-analyses comparing effects of nicotine concentration on outcomes.

For policy makers and practitioners, findings from our review for this chapter suggest that developing and implementing policies and interventions that support smokers to completely switch from smoking to vaping will reduce exposure to toxicants and carcinogens that have links with poorer cardiovascular health.

Chapter 12: Other health outcomes

Evidence reviewed

In this chapter, we address health outcomes not covered in the chapters on the main causes of smoking-related illness and death. From our systematic review, we identified 15 studies in humans that looked at outcomes related to dental health. We also identified 14 studies in humans, 31 in animals and one in cells that investigated other health outcomes.

Studies in humans have assessed associations with a range of health outcomes including oral, ocular and reproductive health, as well as outcomes related to allergies and pre-diabetes. The health outcomes assessed covered a limited range; all were detrimental to health and none of the included studies explored potential positive effects of nicotine or vaping. For instance, no study looked at the effects on Parkinson's disease, where some have suggested a protective effect of nicotine.

Main findings

Limitations of the evidence

Many studies found that health outcomes for people who vaped were worse than for people who did not vape (or did not smoke) while others found no differences. However, while some studies included large samples, they were almost exclusively cross-sectional in design, making any causal statements impossible.

Studies used a range of different definitions of vaping and smoking. For example, findings of some studies were confounded by categorising vapers who smoke, occasional vapers or exclusive daily vapers as a uniform group or comparing occasional vapers with daily smokers. So, findings need to be cautiously interpreted. Definition of user groups, information on and comparisons with smoking were often lacking or confounded the findings.

Many studies were at risk of bias and other factors (for example, genetic, lifestyle and environment) influencing health outcomes were often not considered, further limiting the validity of findings.

Reproductive health

The evidence base on reproductive health or pregnancy outcomes remains insufficient. Previous reports only found a single study indicating that vaping in pregnancy had little or no effect on birth weight. We were not able to add further evidence to these.

Oral or dental health

Oral or dental health has been researched more extensively than other health areas However, the quality of the studies was often low. Recent reviews concluded that vaping would be detrimental to oral or dental health among people who have never vaped or smoked but would likely be beneficial for smokers switching. We found no studies that would change that conclusion.

Cell and animal studies

The one cell and 31 animal studies provided insights into how vaping products may affect the central nervous, digestive and reproductive systems. They also looked at other areas that exposure to tobacco or no exposure could affect. However, the data are still limited and too inconsistent to evaluate the compounds of vaping product aerosol causing any alterations to systems in the body. Also, variability of animal models, exposure methods and comparators added to the uncertainty.

Implications

Good quality studies in humans are needed that investigate the effects of vaping on a wider range of physical and mental health outcomes. They should also explore the progression of various health disorders in people who vape compared with people who smoke or do not vape nor smoke.

Also, although cancer, respiratory and cardiovascular diseases are the main contributors of tobacco related disease, there is a lack of research on the effects of vaping on other areas, such as renal and hepatic systems, which can be greatly affected by smoking.

Effects of vaping on foetal development and pregnancy outcomes remain in particular need of research, including the effects of switching from smoking to vaping in the perinatal phase.

Chapter 13: Poisonings, fires and explosions

Main findings

Poisonings

In 2021, the National Poisons Information Service (NPIS) reported that they had received 187 vaping product enquiries out of a total of 39,594 telephone enquiries. Of these, 82 involved children aged 5 years or younger. This equates to at least one telephone enquiry every other day involving a healthcare professional managing someone who has apparently been exposed to vaping products.

Two case reports of poisoning from vaping products in the UK were identified, both intentional. In one of the cases, the person died.

In non-UK poisonings, according to data from a 2020 annual report by the American Association of Poison Control Centers' National Poison Data System, one person died from vaping product use (no details were given of the circumstances). In 20 studies from international poisons and surveillance centres and case reports identified in our systematic review, most participants were young children who accidentally swallowed e-liquids. Almost all children recovered, although there were 2 deaths among the children who were accidentally exposed to e-liquid. Where exposure was intentional or unknown, there were reports of 16 deaths (outside the UK).

Accidental ingestion is the most common cause of poisonings, with fewer incidences of other routes such as ocular (eyes) exposure.

Incidents of poisoning in children are often preventable.

Fires

Between January 2017 and October 2021, the London Fire Brigade reported that there were 5,706 fires caused by cigarettes and cigarette lighters. This compared to 15 fires caused by vaping products. No fire related injuries or deaths were reported from vaping related fires, compared with 676 injuries and 46 deaths from cigarette related fires. These findings are similar to those we discussed in our 2018 report.

Explosions

Exploding vaping products can cause severe burns and injuries that require intensive and prolonged medical treatment, especially when they explode in users' hands, pockets or mouths.

Incidents appear to be serious but very rare.

We identified 2 case reports involving 4 people in the UK. One involved an explosion in the mouth while vaping, the other 3 involved explosions when the vaping product was being carried in trouser pockets. No deaths were reported.

There were 23 reports identified outside the UK, from case reports and series or data from burn and surveillance of injury centres. Carrying the vaping product in a trouser pocket was again the most common cause of explosions. One death was reported.

Implications

More research on vaping-related poisonings, fires and explosions

There is a lack of UK research or published case reports on poisonings, fires and explosions involving vaping products. The findings reported here are largely from the US and cannot be assumed to be applicable to the UK given the different regulatory frameworks for vaping products.

More research is needed on the type of vaping product resulting in poisoning, fires and explosions. This would then inform future regulations.

Information on poisonings, fires and explosions should be monitored and reported routinely in publicly available reports by relevant authoritative bodies.

Warnings on labelling and devices

Two explosions were identified as caused by mechanical modifiable tank devices, which do not have inbuilt safety features. So, warnings could be highlighted for users of these products by relevant authoritative bodies.

As well as childproof packaging, regulations should require labelling to reinforce safe storage and away from similar looking medicines, such as eye or ear drops and children’s medicine.

Advice on transporting vaping products and batteries

Additional advice by relevant authoritative bodies could be given on transporting vaping products and batteries for example using specialised containers, to avoid thermal runaway incidents (where a battery discharges all its stored energy at once).

Chapter 14: Heated tobacco products

Main findings

Use of heated tobacco products in England

Among young people aged 11 to 18 in the 2021 ASH-Y survey, 0.9% had tried but no longer used heated tobacco products (HTP) and 0.3% reported currently using HTP.

Among young people aged 16 to 19 in the ITC Youth survey, 1.5% had ever tried HTP but not used them in the past week and 0.7% had used HTP in the past week.

Two-thirds (65.7%) of young people aged 16 to 19 who had ever tried HTP had used it once or up to 10 times only.

Among adults in England, 0.3% in the STS and 0.5% in the 2021 ASH-A survey reported currently using HTP.

The proportion of adults who reported having ever used HTP was 1.8%. It was more common among people aged 25 to 34, women and adults who smoked or vaped.

One third of ever or current adult users of HTP had tried HTP once or twice and 16% of current users (less than 0.1% of adults in England) reported daily use.

Among past year smokers who had attempted to stop smoking, 1.6% reported having used HTP to support their attempt.

Cochrane review

The Cochrane review of HTP for smoking cessation and reducing smoking prevalence reported no studies on HTP used to support cessation of cigarette smoking, so the effectiveness of HTP for stopping smoking remains uncertain.

The Cochrane review found moderate certainty evidence that smokers switching to HTPs have lower exposure to toxicants and carcinogens than smokers continuing to smoke. There was moderate to very low certainty evidence of higher exposure than for people attempting abstinence from all tobacco.

There was some evidence for people improving the amount of air they can exhale from the lungs (FEV1) after switching to HTP compared with continuing to smoke. But there was insufficient evidence of any difference for other biomarkers of harm.

There was insufficient evidence for differences in risk of adverse or serious adverse events between people randomised to switch to HTP, smoke cigarettes or attempt tobacco abstinence in the short-term.

The rate of decline in cigarette sales accelerated after Japan made HTP available. However, it is possible that other factors caused this change. A decline in cigarette sales may not translate to declining smoking prevalence, and changes in Japan may not apply elsewhere.

Implications

Monitoring of HTP uptake among young people and adults should continue.

Research independent of manufacturers is needed into whether HTP help people stop smoking, their safety, and their impact on smoking rates.

Chapter 15: Harm perceptions and communications

Evidence reviewed

This chapter drew on surveys carried out in chapters 3 (vaping among young people) and 4 (vaping among adults) and a systematic review that addressed the following questions:

1. What interventions have been effective in changing vaping harm perceptions?

2. To what extent are vaping harm perceptions predictive of any changes in vaping and smoking behaviours?

Main findings

Young people's harm and other perceptions of vaping in England

Among 11 to 18 year olds, using 2021 ASH-Y data:

- 44.7% accurately perceived that vaping was less harmful than smoking
- 32.4% inaccurately thought that the harms from vaping and smoking were about the same
- 3.6% inaccurately thought that vaping was more harmful than smoking
- 19.3% said they did not know

The proportion of 11 to 18 year olds who accurately thought that vaping was less harmful than smoking declined from 66.7% in 2015 to 43.3% in 2020, and then increased slightly in 2021 to 44.7%. The proportion who did not know has increased from 9.9% in 2015 to 19.3% in 2021.

Among 11 to 18 year olds, inaccurate perceptions that vaping is more or equally as harmful as smoking were similar between young people who currently vaped and those who never vaped. Only half of current smokers aged 11 to 18 years accurately perceived vaping as less harmful than smoking.

Among 16 to 19 year olds (using ITC Youth data), we see slightly different patterns in 2021, with most (62.9%) accurately perceiving vaping is less harmful than smoking. Yet, we also saw:

- 16.8% inaccurately perceived vaping to be equally harmful to smoking
- 10.0% inaccurately perceived vaping to be more harmful than smoking
- 10.0% reported that they did not know

In relation to absolute harms, young people (16 to 19 year olds) rated smoking daily higher on the scale of harm than smoking on some days (88.0% compared with 65.2% rating it 'very' or 'extremely' harmful). However, there was less difference between young people's perceptions of vaping daily and vaping on some days (31.9% and 22.6% respectively). Slightly greater proportions of young people perceived some day or daily vaping as not at all harmful (6.2% and 2.8% respectively) than they did for smoking (both 0.6%). A greater proportion of young people did not know the harms of vaping (about 11.5%) than did not know the harms of smoking (less than 1%).

Half of 16 to 19 year olds perceived vaping to be 'slightly' or 'somewhat' addictive (50.7%), one-third perceived vaping to be 'very' or 'extremely' addictive (31.7%), and few (6.3%) perceived vaping to be 'not at all' addictive, with 11.1% saying they did not know.

Over half of 16 to 19 year olds perceived that vaping makes quitting smoking permanently 'a bit' or 'a lot easier' (60.0%). Many (14.2%) thought it had 'no effect', just under one-tenth (9.6%) perceived that vaping made quitting 'a bit' or 'a lot harder', with 15.9% saying that they did not know.

Overall, just over half of 16 to 19 year olds reported noticing any education campaign or public health message about vaping in the past 12 months (53.0%).

Adult smokers' and vapers' harm perceptions of vaping in England

Among adult smokers in 2021 STS data, just over a third (34.1%) accurately perceived that vaping was less harmful than smoking. But around a third (32.1%) inaccurately thought that the harms from vaping and smoking were about the same, 11.9% inaccurately thought that vaping was more harmful than smoking, and 22.0% said they did not know.

The proportion of adult smokers who inaccurately perceived that vaping was more harmful or equally harmful than smoking has declined since 2020 by 2.9 and 5.6 percentage points respectively. The proportion of smokers who accurately perceived that vaping is less harmful than smoking increased by 5.0 percentage points since 2020 (the first time we have seen an increase in this measure since 2014). However, there seems to be growing confusion about the relative harms of vaping compared with smoking. STS found that the

proportion of adult smokers who said that they did not know whether smoking or vaping was more harmful has more than doubled from 9.5% in 2019 to 22.0% in 2021.

In the ASH-A survey, overall, few (13.9%) current adult smokers and vapers accurately believed that none or a small amount of the risks of smoking were due to nicotine, with:

- 23.9% reporting ‘under half the risk’
- 17.3% reporting ‘around half the risk’
- 26.9% reporting ‘much more than half’ or ‘nearly all’ the risk
- 18.1% reporting that they did not know

There was a notable gradual increase in correct nicotine risk perceptions among adults depending on participants’ experience with vaping. The proportions that correctly reported that ‘none’ or ‘a very small amount’ of the health risks from smoking come from nicotine in tobacco cigarettes included:

- 10.8% of current smokers
- 15.6% of smokers and vapers
- 20.3% of exclusive vapers

Systematic review of vaping harm perceptions

We have included a systematic review of vaping harm perceptions examining interventions to change them, and longitudinal associations with vaping and smoking behaviours.

Interventions to change perceptions

We identified 32 articles (from 29 studies) addressing our first research question:

1. What interventions have been effective in changing harm perceptions?

Studies involved either adults or young people, and addressed:

- relative perceptions of the harms of vaping (compared with smoking)
- absolute perceptions of the harms of vaping or addictiveness (vaping compared to non-use of tobacco or nicotine products), such as the perception that e-cigarettes contain harmful chemicals, cause heart disease or cancer, or that vaping is addictive)
- perceptions of the harms of nicotine (including perceived addictiveness of nicotine)

Of the 32 articles, there were:

- 13 articles (from 10 studies) assessing interventions involving written information about vaping
- 4 studies assessing educational workshops or videos designed to deter vaping
- 5 studies assessing mass media campaigns or advertisements
- 3 studies assessing warning labels and packaging
- 3 studies assessing video games aimed to prevent youth vaping
- 4 studies assessing whether vaping harm perceptions changed after the EVALI outbreak

Our review found that interventions communicating information about the reduced harms of vaping relative to smoking generally increased people's perceptions that vaping is less harmful than smoking. Most of this evidence came from studies of adults.

We also found that interventions communicating information about the absolute harms of vaping (vaping compared to non-use of tobacco or nicotine products) generally increased the perception that vaping:

- is harmful to health
- can lead to developing diseases or other health issues
- is equally or more harmful than (relative to) smoking

Most of these interventions were aimed at young people or young adults specifically to deter them from vaping by providing information about vaping harms.

EVALI increased people's harm perceptions of vaping, including inaccurate perceptions relative to smoking.

Warning labels highlighting that vaping is harmful and addictive generally increased people's perceptions that vaping is harmful to health and is addictive.

Vaping harm perceptions predicting changes in behaviour

We identified 21 studies addressing our second research question:

2. To what extent do vaping harm perceptions predict any changes in vaping and smoking behaviours?

Studies assessed young people, young adults or adults, and assessed associations between vaping harm perceptions and vaping and smoking behaviours.

For vaping among young people and young adults:

- 14 studies assessed associations between vaping harm perceptions and changes in vaping behaviours
- 3 studies assessed associations between vaping harm perceptions and changes in smoking behaviours

For vaping among adults:

- 6 studies assessed associations between vaping harm perceptions and changes in vaping behaviours
- 3 studies assessed associations between vaping harm perceptions and changes in smoking behaviours

Our review found that vaping harm perceptions consistently predicted subsequent changes in vaping behaviours among young people, young adults and adults.

Perceiving vaping as less harmful than smoking predicted subsequent increases in vaping (including starting vaping) among young people and young adults, but also among adults and adult smokers. Conversely, perceiving vaping as harmful was associated with not starting vaping among young people and young adults.

Substantially fewer studies assessed whether people's vaping harm perceptions predicted subsequent changes in their smoking behaviours. However, the limited evidence suggests that perceiving vaping as equally or more harmful than smoking predicted subsequent relapse to smoking among adult former smokers. Also, perceiving vaping as less harmful than smoking predicted quitting smoking. But among young people and young adults, relative and absolute harm perceptions (sometimes including perceived risk of addiction) were not associated with starting smoking. Absolute harm perceptions were not associated with smoking more.

In general, the findings were broadly consistent with people's normal expectations for approaching what they perceive to be lower harm and avoiding what they perceive to be greater harm.

Taken together, the findings suggest that messages about the harms of vaping influence vaping perceptions. This in turn affects people's vaping and smoking behaviours.

Providing information aimed to deter young people from vaping (for example, highlighting the harms of vaping) can increase their perceptions of the harm of vaping to health, which in turn can deter them from trying vaping. Conversely, providing information aimed to increase accurate relative perceptions of vaping compared to smoking can increase accurate relative perceptions of vaping compared with smoking. This could lead adult smokers to try vaping, reduce risk of relapse to smoking among adult former smokers who vape, but it could also lead to young people trying vaping.

The effects of vaping harm perceptions on longer-term vaping, smoking, and vaping as a substitute for smoking, remain unclear.

Risk of bias was high for all included studies for both our research questions.

Implications

The need for carefully designed interventions

Given a substantial proportion of young people and adult smokers and vapers in England still hold inaccurate perceptions of the relative harms of vaping compared with smoking (that vaping is equally or more harmful than smoking), these misperceptions need to be addressed.

Providing accurate information about the relative harms of vaping, and risks of using nicotine, could help to correct misperceptions of vaping and nicotine, respectively, particularly among adults.

Interventions on absolute harms of vaping need to be carefully designed so as not to misinform young people (particularly smokers) about the relative harms of smoking and vaping.

The need for research on effects of warning labels highlighting relative harms of vaping and smoking

Warning labels highlighting that vaping is harmful and addictive generally increased perceptions that vaping is harmful to health and is addictive. No studies assessed the effects of warning labels highlighting the relative harms of smoking and vaping, on relative harm perceptions. So, these studies are needed.

Other research needed

No studies among young people or young adults assessed whether vaping harm perceptions predicted subsequent switching from smoking to vaping, or the other way around. So, studies addressing substituting smoking with vaping in young people, young adults and adults are needed.

More longitudinal randomised studies assessing interventions to change vaping harm perceptions are needed. There is also a need for studies that assess whether changes in vaping harm perceptions (in response to interventions) and vaping and smoking behaviours (associated with harm perceptions) are maintained over time (particularly into adulthood).

Importance of effective communications

Communications about absolute and relative harms of vaping and smoking are likely to reach both young people and adults. From an ethical standpoint, the main aim of these communications must be to ensure that the messages give accurate information about absolute harms of vaping, and the relative harms of vaping compared to smoking, to address the prevalent misperceptions. Messages will need to be carefully developed and nuanced to avoid unintended effects (for example, ‘less harmful’ translating to a perception of ‘safe’) and should be tested on target audiences first. Finally, continued surveillance of perceptions in young people and adults is needed.

Chapter 16: Conclusions

In this chapter, we summarise the findings from each chapter and pull together the above findings in the context of the series of evidence reviews since 2015. We also present the conclusions from the systematic reviews in the form of evidence statements. We then present overall implications for policy, practice and research.

Despite the increase in research on vaping since 2015, weaknesses around the choices of assessments and biomarkers, populations, user groups and exposure, and study designs all limit the conclusions that we can draw.

Overall findings in the context of our series of evidence reviews

We have previously stated, in our 2015 report, vaping poses only a small fraction of the risk of smoking and is at least 95% less harmful than smoking (that is, smoking is at least 20 times more harmful to users than vaping). This was to help the public and health professionals make sense of the difference in the magnitude of risk between vaping and smoking.

We are aware that summarising the relative risks of vaping versus smoking across a range of different products and behaviours and assessed across multiple biomarkers can be simplistic and misinterpreted. Based on the reviewed evidence, we believe that the “at least 95% less harmful” estimate remains broadly accurate, at least over short term and medium term periods. However, it might now be more appropriate and unifying to summarise our findings using our other firm statement: that vaping poses only a small

fraction of the risks of smoking. As we have also previously stated and reiterate, this does not mean vaping is risk-free, particularly for people who have never smoked.

This magnitude of relative risk between vaping and smoking is not reflected in current public perceptions which, as our review has shown, can be influenced by interventions.

Evidence statements

In the chapters which reported on our systematic literature reviews of the health risks of vaping and harm perceptions, and the 2022 Cochrane review on heated tobacco products, we listed 61 evidence statements. These statements are based on the strength of evidence, given the quality of the studies we reviewed and their findings. The statements broadly follow the definitions of level of evidence in the NASEM report. As NASEM noted, the framework is a guide, but a great deal of expert judgement, in our case by the co-authors of our report, is also involved.

Recommendations for research

We made a number of recommendations for research. These included:

- involving people who currently smoke or vape to help shape and design research to ensure research questions are relevant, interpret the evidence and support dissemination
- agreeing a common set of biomarkers of exposure and potential harm to be used
- standardising the definitions of who is involved in the research, their exposure to vaping and smoking, and how studies report details of the devices involved
- agreeing protocols for the different designs of studies used
- greater transparency to reduce bias in research, for example pre-registration of study protocols and analytical plans

Overall implications

Evidence from stop smoking services and the Cochrane living review for smoking cessation (not covered in our report) shows that vaping is effective for stopping smoking. These findings, along with our findings that vaping carries a small fraction of the health risks of smoking, suggest that smokers should be encouraged to use vaping products (or medicinally licensed products) for stopping smoking, or as alternative nicotine delivery devices to reduce the health harms of smoking.

Our findings of higher absolute exposure to toxicants from vaping, compared with not using any nicotine products, reinforce the need to discourage people who have never smoked from taking up vaping (or smoking). Cuts to government bodies responsible for overseeing vaping products are concerning. The recent increase in young people using disposable vaping products makes this an even greater concern, because if it continues, it could undermine the approach and regulatory framework for vaping products adopted in England.

As well as educational materials aimed at older smokers on why and how to vape to stop smoking, educational materials are also needed for young people starting vaping who would otherwise not have smoked, and for those who need support in stopping smoking.

It is vital that surveys that assess smoking and vaping are adequately resourced and maintained over time to enable long term trends to be assessed. For example, it would be useful for the Adult Population Survey to include questions about nicotine vaping product use, given the prevalence of vaping.

Public perceptions of absolute and relative vaping harm are not in line with the evidence and our findings indicate that these perceptions influence subsequent vaping and smoking behaviours. We also found that interventions can influence perceptions. So, understanding and changing misperceptions is very important.

Systematic reviews are resource intensive, and since our July 2021 cut-off date for searching the relevant literature for the health chapters, new studies have been published. Future evidence reviews of the health harms of vaping should adopt a continual approach to updating the literature, similar to the living systematic review for e-cigarettes for smoking cessation by the Cochrane Tobacco Addiction Group. This would ensure that relevant new evidence would be incorporated as it becomes available, and would help policy makers to use the most up-to-date evidence.

1 Introduction

1.1 Objective of the report

This report is the eighth in a series of independent reports commissioned formerly by Public Health England (PHE) (1 to 7) and now the Office for Health Improvement and Disparities (OHID) in the Department of Health and Social Care (DHSC), to summarise evidence on vaping products to inform policies and regulations. Alternative nicotine delivery devices such as vaping products can play a critical role in reducing the enormous health burden caused by cigarette smoking, which remains the largest single risk factor for death and years of life lived in ill-health and a leading cause of health inequalities in England, and the second most important risk factor for death and Disability Adjusted Life Years globally (8). However, the impact of nicotine vaping products will depend on how much they displace smoking completely, including among disadvantaged smokers, the extent of uptake among young people, and the absolute health effects of vaping, as well as the relative health effects compared with smoking.

This current report focuses predominantly on the potential health risks of vaping. We carried out a systematic review of the health risks of vaping and divided the findings into chapters covering: biomarkers of exposure to nicotine and potential toxicants; biomarkers of potential harm to health cutting across several diseases; biomarkers specific to cancer, respiratory disease and cardiovascular disease; poisonings, fires and explosions; nicotine and flavours; and then a chapter covering other health outcomes. This report also covers the latest evidence on prevalence and characteristics of vaping in young people and adults in England, with a focus on data emerging since our last report published in early 2021 (5), prevalence of heated tobacco product use in England, incorporating a summary of the latest Cochrane Review on heated tobacco products (9), and a new systematic review on harm perceptions of vaping products and interventions to affect the perceptions.

1.2 Terminology

This report explores nicotine vaping. The term ‘vaping products’ used in the report describes e-cigarettes and refill containers (e-liquids) intended for nicotine vaping. Some vaping products do not always contain nicotine - where studies explored products without nicotine, we refer to them as non-nicotine vaping/vaping products.

The term ‘vapers’ in the report refers to people who use vaping products and ‘vaping’ as the act of using a nicotine vaping product.

We refer to non-users’ exposure to vaping emissions as ‘secondhand vaping exposure’.

These terms do not include cannabis vaping or the vaping of other legal or illegal substances, which are not the subject of this report.

1.3 Vaping products

Vaping products are manufactured by tobacco industry companies and companies independent of the tobacco industry. A recent paper using 2019 survey data indicated that just over half (53%) of respondents who vaped used a tobacco industry product (10). Vaping products come in a variety of shapes and sizes which can be broadly categorised as:

1. Disposable vaping products: one-time (single use) products.

2. Cartridge or pod vaping products: reusable, rechargeable kits designed with replaceable cartridges or pods.

3. Tank vaping products: reusable, rechargeable kits designed to be refilled with liquid by the user. These are often referred to as tanks, sometimes refillable devices; there are also refillable pods available.

4. Modular vaping products: reusable, rechargeable kits often referred to as ‘mods’ (modifiables) that allow users to customise their product such as by regulating the power delivery from the batteries to the heating element (sometimes these are included with other tank models).

Survey data also showed that tobacco industry vaping products were less likely to be refillable types (10).

1.4 Current vaping regulations in England

As detailed in our previous reports, non-nicotine containing vaping products fall under the General Product Safety Regulations 2005, enforced by local authority trading standards. Nicotine vaping products are largely regulated by the European Union Tobacco Products Directive (2014/40/EC) (EU TPD), transposed into UK law by the Tobacco and Related Products Regulations 2016 (TRPR). The national competent authority for the TRPR regulations relating to vaping products is the Medicines and Healthcare products Regulatory Agency (MHRA), acting for the Secretary of State for Health and Social Care. A post implementation review was conducted by the government in January-March 2021 and published in March 2022; this is discussed below.

Table 1 adapted from our previous reports gives a brief overview of the current regulations pertaining to nicotine vaping products in the UK. The regulations are largely similar to

those in the European Union (EU). The most up-to-date advice on regulations for consumer nicotine vaping products is in the MHRA's E-cigarettes: regulations for consumer products.

Summary of the UK nicotine-containing vaping product regulations

The following summary has been adapted from Vaping in England: an evidence update including mental health and pregnancy, March 2020: a report commissioned by Public Health England (3).

Notification requirements

Vaping product manufacturers must submit a range of details to MHRA before putting a product on the market and update when products are manufactured or withdrawn

Maximum capacities and nicotine strength allowed

Tank capacity: 2mL

E-liquid refill container capacity: 10mL

Strength of e-liquid: 20mg/mL

Other safety and quality standards

Child-resistant and tamper evident packaging

Prohibition of certain additives such as colourings

Protection against breakage and leakage, and a mechanism for ensuring re-filling without leakage

Information provision

Health warning and provision of information on pack or device/bottle

Advertising

All broadcast media and cross-border advertising prohibited

Domestic advertising allowed such as outdoor, posters, cinema, etc

All advertising must adhere to a Committee of Advertising Practice Code

Health claims on advertising are allowed under strict conditions

Age of sale law

18 years minimum age and proxy purchasing also prohibited

Public places

No legislation but local proprietors or organisations can decide

Taxation

20% VAT (substantially lower than tax on tobacco products)

1.5 MHRA safety monitoring

Vaping products notified to the MHRA

The MHRA has a public facing database of products that have been notified including a list of withdrawn notifications. There were over 8,000 notifications made using the new system put in place following the UK's exit from the European Union on 1 January 2021. A historic list of previously notified products published for supply in Great Britain prior to 1 January 2021 is also available from the same website, some of which are still legal to supply. There may also be some overlap between the 2 lists.

Retailers are advised to check these lists when sourcing new supplies of any vaping product or vaping liquid. Consumers can also check these lists if interested.

A study published in 2021 by Nyakutsikwa and others at the University of Nottingham in England (11), analysed data reported to the MHRA via the EU Common Entry Gate system in the first year of operation (from November 2016 to October 2017). During this period, 40,785 e-liquid-containing products were notified to the MHRA. Reports were not standardised in relation to units of measurement or constituent terminology.

The mean volume of e-liquid was 10.1mL, and products listed an average of 17 ingredients. Just over half the products (59%) contained under 12mg/mL nicotine with a small minority (less than 1%) having nicotine concentrations above the legal limit of 20mg/mL. More than 1,500 ingredients were identified, of which the most common 6 non-flavours and 38 flavours were identified in more than 10% of products. Flavourings identified are discussed in chapter 6 on flavours. The most common non-flavours other than nicotine were propylene glycol (97.5% of products), glycerol/vegetable glycerine (71.0%), water (34.7%), glycine (33.1%) and ethanol (26.3%), that were typically categorised as carriers. A number of heavy metals, present in no more than 0.01% of products, including iron, zinc, nickel, lead and titanium, were listed in the database. The most frequently reported emissions were nicotine (65%) formaldehyde (48%), acetaldehyde (40%), acrolein (31%) and diacetyl (6%). Most common emissions, other

than nicotine or those listed as carriers, were present in median estimated concentrations for the most part, below published safe limits for ambient air (12, 13).

Nyakutsikwa and others (11) suggested that their findings were cautiously optimistic that in most cases, products notified to the MHRA in 2016 to 2017 were unlikely to cause serious long-term harm, and that it identified opportunities to minimise the potential hazards of e-liquid -containing products on the UK market by both imposing a standardised reporting system so that analysis can be more inclusive, and by acting to bring down emission levels to below likely safe limits.

Analysis of this database is very time-consuming and laborious. Products will have changed somewhat since 2017 and it would be helpful for surveillance purposes if an analysis of notified products could be implemented biennially, funded by government.

MHRA Yellow Card scheme

As discussed in previous reports, the MHRA runs a Yellow Card reporting scheme for vaping products.

The MHRA's Yellow Card scheme collects and monitors information on safety concerns or incidents involving medicines or medical devices in the UK. It is based on voluntary reporting by health professionals and the public. Accordingly, anyone can report an adverse reaction that they suspect to be related to vaping. The reports are therefore not evidence of a proven side effect nor of a causal link between vaping and the suspected adverse reaction. The MHRA's Yellow Card scheme comprises all reports submitted by consumers, healthcare professionals as well as those reported by industry. In 2019, the MHRA had requested that all suspected respiratory reactions that had been reported to industry were shared with the MHRA, meaning that data from the Yellow Card scheme are not directly comparable over time.

The purpose of this reporting scheme is to enable regulatory action to be taken as appropriate and in response to its assessment of those reports. Alongside national regulations, the MHRA also works with local Trading Standards teams to investigate concerns that might relate to specific products (see below).

A data request to the MHRA identified that as of 13 January 2022 and since the Yellow Card scheme was put in place for vaping products on 20 May 2016, it had received 257 Yellow Card adverse reaction reports covering 720 adverse reactions.

The MHRA with its internal team of expert medical assessors determines the seriousness of a report based on whether the reaction term is considered serious in the medical dictionary, MedDRA (14), which is used to code all adverse reaction reports, and the Council for International Organisations of Medical Sciences (15) seriousness criteria. The

MHRA also allows a reporter to state that they consider a report serious for another reason.

Between 1 January 2021 and 13 January 2022, 14 of the reports were considered serious (resulting in a total of 122 serious reports since May 2016) and 12 non-serious (resulting in a total of 135 non-serious reports since May 2016). There were no fatalities reported between 1 January 2021 and 13 January 2022.

No new specific requests to industry were made for respiratory disorder reactions since our last report (5) and no reports were received from the industry.

A new National Institute for Health and Care Excellence (NICE) guideline published on 30 November 2021 (16) (see below) recommended that health professionals ask adults who use nicotine-containing vaping products about any side effects or safety concerns that they may experience, report these to the MHRA through their Yellow Card scheme and also advise people that they can report side effects directly.

Table 1. MHRA Yellow Card reports of suspected adverse reactions associated with nicotine-containing vaping products received between 20 May 2016 and 13 January 2022

Reaction name	Number of reactions
Blood disorders	1
Cardiac disorders	15
Ear disorders	4
Endocrine disorders	1
Eye disorders	7
Gastrointestinal disorders	90
General disorders	86
Immune system disorders	18
Infections	13
Injuries	16
Investigations	5
Metabolic disorders	3
Muscle and tissue disorders	9
Nervous system disorders	50
Pregnancy conditions	1
Product label/physical/quality issues	32
Psychiatric disorders	9
Respiratory disorders	337
Skin disorders	20
Vascular disorders	3
Total reactions for drug	720
Total reports (a)	257
Total fatal outcome reports	3(b)

Table notes: (a) The number of reports is lower than the total reactions because each report constitutes an individual for whom more than one adverse reaction could have been reported. (b) There were no fatalities reported during this period; three suspected fatalities had been reported in 2019 to 20 and discussed in our previous report.

Adverse reactions for licensed stop smoking medicines

The MHRA also publishes information about suspected adverse effects of nicotine replacement therapy and varenicline in the form of interactive Drug Analysis Profiles (iDAPs). For context, from January to December 2021 the iDAP for nicotine replacement therapy included 601 reactions from 297 reports, and no fatalities; the iDAP for varenicline in the same reporting period included 153 adverse reactions from 78 reports, with no fatalities.

Varenicline recall

In June 2021 Pfizer, the sole supplier of Champix (varenicline) in the UK temporarily stopped its distribution after it found presence of nitrosamines (N-nitroso-varenicline) in its products. The MHRA initially issued an alert about the disruption to supply in June 2021 and a recall of Champix in October 2021 (17).

The MHRA's recall was a precautionary measure due to presence of levels of N-nitroso-varenicline above the acceptable level of intake set by both the European Medicines Agency and MHRA. Since this time health professionals have not been able to prescribe Champix and were advised to switch patients to nicotine replacement therapy or bupropion.

1.6 Medicinal nicotine vaping products

There are still no nicotine vaping products licensed as a medicine and available on the market. As outlined in our 2018 report (6), licensed vaping products would be exempt from the Tobacco and Related Products Regulations 2016 (TRPR) and subject to medicinal regulations instead. For example, this would enable higher nicotine content, the products to be promoted for smoking cessation, and would enable health professionals to prescribe the products, including to more disadvantaged smokers.

The MHRA guidance for medicinal vaping products was first published in 2017 but since that time, no licensed vaping products had been brought to market. In our last report (5), we stated that in December 2018, the MHRA announced it would convene an Ad Hoc Working Group for E-cigarettes which met in 2019 to 2020. On 29 October 2021, the MHRA published updated guidance for companies wishing to apply for licensing of a vaping product as a medicine (18). The updated guidance was intended to clarify the

requirements for licensing, and at its launch, the MHRA indicated it was encouraging manufacturers to come forward with applications to license vaping products.

Dr June Raine, Chief Executive of the MHRA, stated at the launch of the updated guidance:

> "The updated guidance on licensing requirements we have published today is a strong first step towards availability of safe and effective licensed e-cigarette products. The MHRA will continue to support companies in the development of safe and effective e-cigarette products, to encourage the licensing of e-cigarette products as medicines in order to support patient-centred care and access" (18).

The changes predominantly focused on quality standards for dose uniformity, non-clinical toxicological data requirements and the design of clinical pharmacokinetic studies, in addition to changes necessitated by the UK leaving the EU. It shortened review timelines to 80 days for review and 150 days for targeted decisions.

Since that time, however, we are aware that concerns have been raised that these revised timelines are not being met. We understand that the MHRA are giving the issue greater priority but we consider that it is likely that the planned restructuring of the MHRA may exacerbate the situation (19, 20).

1.7 Local authority trading standards

Trading standards officers have a sub-regional footprint working in local authorities. They enforce consumer legislation in their local areas, which includes advice on consumer law, such as consumer safety and counterfeit goods, investigating complaints and prosecuting traders who break the law.

In previous reports we have summarised surveys of tobacco control activities in local authority trading standards services in England, carried out by the Chartered Trading Standards Institute (CTSI). However, there have been no new surveys in the last two years due to a combination of the COVID-19 pandemic and reduced financial resources. Our understanding is that there are currently no plans to reinstate it.

Instead, we report findings from a national project in Scotland, conducted by the Society of Chief Officers of Trading Standards in Scotland (SCOTSS) and the National Tobacco and Age Restricted Products (TARP) Groups (21). The national project was in response to information from trading standards throughout the UK that illegal single use nicotine containing vaping products were being sold in retail premises and were being used by people under the age of 18, often inside schools. The disposable vaping devices of concern are single use products that are a brightly coloured sealed unit which are similar

in appearance to a large highlighter pen. They usually contain nicotine salt liquid and a lithium-ion battery and come in a wide variety of flavours. They are designed to be thrown away once they run out of charge or e-liquid.

SCOTSS and TARP coordinated surveillance of stock and enforcement activities in 21 local authorities between 15 October and 24 December 2021 and collated the outcomes. A total of 721 retail and wholesale premises were visited; 88,839 disposable vaping devices were removed from sale. It is not reported what proportion of devices this represented. These were either not labelled correctly in accordance with the TARP Regulations, did not contain sufficient Classification, Labelling and Packaging EU regulation information and approximately 70% of all products and brands found during the project had not been approved and published by the MHRA. Where they had been notified, 89 unique products included the word ‘energy’ or ‘lite’ in the name, which is prohibited under TRPR. Of the non-compliant devices, 3,683 had a capacity of over the legal limit of 2mL. All the discovered devices were imported from China.

Some authorities in Scotland also conducted compliance testing of age of sale verification. Retailers are obliged to have an age verification policy which states that they ask anyone attempting to buy a nicotine containing product who looks under the age of 25 for photographic proof of age. Between 1 August 2021 and 31 January 2022 there were 36 recorded incidents relating to the supply of vaping products to under 18 year olds and a further 9 related to the supply to under 18s who had not been asked for any proof of age during integrity testing projects run by Aberdeen City and North Lanarkshire Council.

The report recommended that:

- disposable vaping devices should be inspected by trading standards at their point of entry into the country
- the monitoring of the market should be conducted on behalf of the Secretary of State for Health and Social Care by the Office of Product Safety and Standards (OPSS) and MHRA
- the regulations relating to waste batteries in particular regarding the disposable vaping device market, should be enforced by the OPSS
- including inspection of the packaging and the disposable vaping devices as part of the notification process conducted by the MHRA would be a positive step in controlling this market
- MHRA should add definitive advice to the electronic cigarette information pages on their website

There are numerous reports in the press and on council websites in England of seizures of illegal disposable vaping products (due to similar reasons described above), but as yet there is no formal report pulling this information together across England which would help to give a national perspective of this issue. However, a study of underage access to vaping products is currently being conducted by the Chartered Trading Standards Institute in England.

1.8 Advertising

We reported previously that the blanket ban on health claims on permitted forms of vaping product advertising (domestic channels) was lifted in November 2018, but that the guidance for health claims states that they need to be product-specific and supported by evidence (3). To our knowledge, no marketers for vaping products have made a health claim. These conditions should therefore be part of the proposed review of vaping product regulations (see below).

On 31 March 2021 Cancer Research UK published a report (22) examining the marketing of vaping products in the UK bringing together 2 complementary studies covering advertising spend, advertising content, compliance with advertising regulations and survey data on noticing and appeal of marketing among adults between 2016 and 2018 and youth between 2017 and 2019 across England, the US and Canada.

Findings included that:

- almost all of vaping product advertising expenditure was in permitted channels in 2019 and that compliance with the advertising code (see summary of the UK nicotine-containing vaping product regulations above) was generally high in a sample of the advertisements which were studied

- expenditure was not available for point of sale and social media marketing channels but in a sample of Instagram posts studied for compliance, all were found to be in violation of the code

- the proportion of young people who had never smoked or vaped noticed marketing for vaping products at consistently higher rates than adults who smoked

- there were high rates of noticing across social media despite it being a prohibited channel

- small increases in youth noticing vaping product marketing in allowed channels

- increases in youth perceptions that vaping product advertisements targeted people who did not smoke

- cross-country comparisons suggested that vaping product marketing regulations in England were limiting exposure to both adults and youth

These findings suggested that the current regulations might need adjustments to balance between marketing targeting adults who smoke, but not reaching young people who are unlikely to ever take up smoking or vaping.

Concerns have been expressed about vaping product packaging and the use of imagery that will attract youth such as cartoons (23). These issues were also picked up by the All Party Parliamentary Group on Smoking and Health recommendations for the new tobacco control plan and the Action on Smoking and Health (ASH) and SPECTRUM submission to the post implementation review of the TRPR which are discussed further below.

1.9 UK government strategies, consultations and relevant commissioned work

Tobacco control plan for England

A new tobacco control plan, initially announced for 2021, has not yet been published. The ambitions set out in the 2017 tobacco control plan for England (24) therefore currently remain in place. In our 2021 report (5), we discussed the progress made towards the ambitions set out in the 2017 tobacco control plan and below we revisit this with the latest data.

Ambition 1: The first smokefree generation

“People should be supported not to start smoking, so we aim, by the end of 2022 to:

- reduce the prevalence of 15 year olds who regularly smoke from 8% to 3% or less.
- reduce smoking prevalence among adults in England from 15.5% to 12% or less.
- reduce the inequality gap in smoking prevalence between those in routine and manual occupations and the general population.”

Prevalence of 15 year olds who regularly smoke was to be assessed by the Smoking, Drinking and Drug Use Survey; the latest data available are still from 2018 which were 5% (25). Prevalence of smoking among adults in England was to be assessed by the Annual Population Survey; in 2019, smoking prevalence was 13.9% (26). For 2020, data collection was disrupted due to COVID-19 and data have been published separately for the first quarter (13.5%) and later quarters (12.1%) due to a change in data collection modality which reduces comparability of prevalence figures (27).

Reducing the inequality gap in smoking prevalence between those in routine and manual occupations and the adult population as a whole can be assessed by the Office for National Statistics (ONS) from the Annual Population Survey – 26.5% in the routine and manual category (2016) compared to overall adult smoking prevalence of 15.5% (2016), working out as a ratio of 1.7; in 2019, prevalence was 23.4% in the routine and manual category compared to adult smoking prevalence of 13.9% (26), again a ratio of 1.7. Using data from April to December 2020 with the caveat of the different data collection modality, smoking prevalence was 21.4% among those in routine and manual occupations, 1.8 times the prevalence of 12.1% in the overall adult population, which shows no improvement compared with data from 2016 and 2019.

Ambition 2: A smokefree pregnancy for all

“Every child deserves the best start in life, so we aim, by the end of 2022 to:

- reduce the prevalence of smoking in pregnancy from 10.7% to 6% or less.”

Prevalence of smoking in pregnancy was to be assessed by the NHS Digital Smoking Status at Time of Delivery data; in the first 2 quarters of 2021 to 2022 (April to September 2021), 9.1% of women were recorded as smoking at the time of delivery (28, 29).

Ambition 3. Parity of esteem for those with mental health conditions

“People with mental ill health should be given equal priority to those with physical ill health, so we aim to:

- improve data collected on smoking and mental health to help us to support people with mental health conditions to quit smoking

- make all mental health inpatient services sites smokefree by 2018.”

As we reported in our last report (5), the improvement in data collected on smoking and mental health to help support people with mental health conditions to quit smoking is variable. There are now better data about smoking prevalence from the annual General Practice Patient Survey. There have also been improvements in data collection about smoking status and the provision of brief advice for people in inpatient mental health settings through the Preventing ill health by risky behaviours: alcohol and tobacco Commissioning for Quality and Innovation (CQUIN) indicator. However, data collection in community mental health settings remains poor.

We also previously reported (5) that in relation to the ambition to make all mental health inpatient services sites smoke-free by 2018, a survey by ASH in 2019 found that 37 of 45 mental health trusts that responded to the survey (82%) prohibited smoking on all trust

premises (30). Note there are 54 mental health Trusts in England overall. A more recent survey has not been carried out.

Ambition 4: Backing evidence-based innovations to support quitting

"We are committed to evidence-based policy making, so we aim to:

- help people to quit smoking by permitting innovative technologies that minimise the risk of harm.

- maximise the availability of safer alternatives to smoking."

In chapter 4 (vaping among adults) of this report we discuss the proportion of quit smoking attempts that are made using a vaping product. As reported previously (5), vaping products are widely available in England, but perceived relative availability compared with tobacco cigarettes has not been assessed to our knowledge. Also, we comment on the lack of a licensed vaping product above, which might increase accessibility to more disadvantaged smokers.

In conclusion therefore, the smokefree mental health services ambition appeared to have been missed in 2018, the smoking in pregnancy ambition looks very unlikely to be met, and the inequality gap does not appear to be reducing. The adult and youth ambitions appear potentially achievable although continuing COVID-19 disruptions to routine surveys make assessment of these difficult.

Advancing our health: prevention in the 2020s

In our 2021 report (5), we discussed the government's Green Paper consultation document published in July 2019, 'Advancing our health: prevention in the 2020s' (31). In this Green Paper, the government stated that its ambition was to go smoke-free in England (smoking prevalence at 5% or below) by 2030. For clarification, vaping is not included in smoking prevalence and so the smokefree target does not include vaping.

The Green Paper included an ultimatum for industry to make smoked tobacco obsolete by 2030, with smokers quitting or moving to reduced risk nicotine delivery systems such as vaping products. It invited ideas on ways to raise additional funding for tobacco control, such as a levy on tobacco companies. Although the new tobacco control plan to deliver the smokefree 2030 ambition was originally due to be published in July 2021, we understand that this is now due to be published in 2022.

Post Implementation Review of the TRPR

In our last 2 reports (3, 5) we highlighted that the UK government had committed to review the TRPR (32) by 20 May 2021 to assess whether the regulations had met their

objectives. This Post Implementation Review was published in March 2022 based on a review of commissioned evidence, published peer-reviewed evidence, responses to a public consultation and assessment of key indicators (33). The DHSC concluded that the TRPR regulations had met their original objectives and they could not be better achieved through alternative regulatory measures.

Some areas with potential for amendments were identified which included some relevant to vaping products, derived mainly from stakeholder comments. Some stakeholders raised concerns that the nicotine strength limits were not high enough to help some smokers switch permanently to exclusive vaping and help the government achieve its smokefree 2030 ambition. In addition, many from industry, other organisations and vapers felt the tank size limits, and bottle sizes should be increased as they were inconvenient. In terms of the TRPR requirements for vaping product warning messages, the review included a study suggesting that they may deter smokers from switching to vaping products. Some stakeholders also wanted stronger regulations for vaping products in terms of restricting the packaging and descriptor names to protect youth from using these products. There were also some other tobacco and nicotine products that the regulations did not cover, with some suggesting they should do. For example, some stakeholders thought the non-nicotine vaping industry should be regulated in the same way as nicotine vaping and that nicotine pouches and other novel nicotine products should also be regulated under the TRPR regulatory framework. This would improve standards and consumer safety, and ensure regulation was coherent. Overall, the evidence presented was seen as providing a strong argument for retaining the regulations. However, the government indicated it would consider the proposed amendments made by stakeholders when considering any further regulatory reforms necessary as part of its plans to meet the smokefree 2030 targets, but that any changes would be based on ‘robust evidence and support improvements to public health’.

The All-Party Parliamentary Group on Smoking and Health recommendations for the Tobacco Control Plan 2021

In our last report (5) we discussed the Roadmap to a Smokefree 2030 which was published by the Smokefree Action Coalition (34). In June 2021, a group of parliamentarians lent their voices to this debate: the All Party Parliamentary Group (APPG) on Smoking and Health published a set of recommendations for the new Tobacco Control Plan (35). The APPG on Smoking and Health is a cross-party group of Members of Parliament and Peers which was founded in 1976 and its secretariat is provided by the charity ASH.

Indicating that at current rates of decline the smokefree 2030 target would be missed by 7 years, and by 14 years for the poorest in society, the APPG stated that investment would be required (and recommended this be from the tobacco manufacturers), but that the benefits would outweigh the costs. The APPG believed that the evidence about what

policy levers work was clear, but that these levers needed to be pulled by government to their fullest extent. The APPG set out 12 recommendations which they indicated needed to be put in place by the end of 2021 and sustained until at least 2030. Three recommendations covered setting the course for smokefree 2030 which included funding and the need to set interim targets for 2025. Five recommendations urged to level up through targeted investment, such as delivering anti-smoking behaviour change campaigns targeted at routine and manual and unemployed smokers.

The final 4 recommendations focused on shaping the consumer environment and included 2 relevant to vaping products:

- reduce the appeal and availability of e-cigarettes and other nicotine products to children

- make the route to medical licensing fit for purpose to allow e-cigarettes to be authorised for NHS prescription

In relation to reducing appeal and availability to children, the APPG indicated that there were loopholes in the current legislation which needed closing. They mentioned that non-nicotine vaping liquids could be sold legally to children and there was no restriction on the volume of these liquids nor how they were packaged. These are sometimes referred to as ‘short-fills’ and are then combined with nicotine shots which are sometimes given away for free meaning that all these products can be accessed by children relatively easily. We first brought attention to the issue of ‘short-fills’ in our 2018 report (6) and free vaping products to youth in our 2021 report (5). The APPG highlighted ASH research suggesting that restricting packaging designs would reduce the appeal of vaping to young people while having little impact on adult smokers’ interest in using the products to quit smoking; research on this is ongoing at King’s College London in collaboration with ASH and the University of Waterloo in Canada. The second loophole was in relation to advertising of vaping products on social media through channels such as Instagram and TikTok and the APPG recommended that a review of enforcement processes be carried out to strengthen the regulations. Overall, the APPG recommended that packaging and labelling shown to appeal to young people be prohibited, such as product names or descriptors such as sweet names (for example ‘gummy bears’), and attractive colours or cartoon characters on packs. The APPG further recommended prohibiting free distribution; and review and revise as necessary the current warning on vaping products to ensure its effectiveness in deterring youth while not deterring use by adult smokers.

In relation to licensing vaping products, the APPG recommended that a licensed product be available on the market by the end of 2022. It called on the MHRA to update its licensing guidance and commit to providing support and feedback to companies preparing applications (which has since been done as discussed above).

Post implementation review of the Nicotine Inhaling Products (Age of Sale and Proxy Purchasing) Regulations 2015

A report on the Nicotine Inhaling Products (Age of Sale and Proxy Purchasing) Regulations 2015 was published in January 2021 as part of a post implementation review report of tobacco legislation coming into force between 2010 to 2015 (36)

The policy objective of the regulations is to limit the sale of nicotine inhaling products to adults only, to limit the availability of nicotine for young people and minimising young people becoming addicted to nicotine. The regulations do not apply to tobacco products which were already covered by age of sale legislation. The review concluded that evidence and data available to assess this regulation were limited. Vaping prevalence among young people has declined slightly since 2016, indicating that the regulation has served to check any potential growth in vaping product use. It was noted that adult prevalence over the same period has continued to increase. The conclusion of the post implementation review cited our 2018 review as also showing no evidence of vaping acting as a gateway into smoking tobacco, to which the regulations limiting sale of nicotine inhaling products to adults likely played a role. Overall, the review concluded that the legislation had achieved its original objective.

Independent review into tobacco control

In February 2022, as part of its work to tackle health inequalities, the UK government launched an independent review into tobacco control in England (37). Javed Khan OBE, former CEO of children's charity Barnardo's would review the government's 2030 smokefree ambition and seek to identify the best ways to address health inequalities caused by smoking. The review would cover smoking prevention and cessation interventions and how both can be improved to support those who experience the biggest harms from smoking. The findings were to inform the government's new Tobacco Control Plan discussed above.

Summary of UK situation

The new Tobacco Control Plan for England will set out the government's strategy to reach the smokefree 2030 target. This will need to be more ambitious than the previous plan and be comprehensively funded if the target is to be met. The focus on inequalities is critical given the persistent inequalities in smoking prevalence.

As in our previous review (5), we consider it appropriate that all aspects of vaping products (and other non-tobacco nicotine products) be reviewed at this stage, in particular:

- regulation of non-nicotine vaping products given they are governed by different regulations and bodies to nicotine vaping products

- regulation of other non-tobacco nicotine products such as nicotine pouches which entered the market after the implementation of the TRPR
- limits set on product characteristics, such as nicotine content, containers and tanks
- advertising restrictions
- labelling and packaging requirements
- regulations around harm reduction claims and validation
- licensing process for nicotine vaping products given no such products are yet available
- availability of products (free samples) and ease of purchasing by young people despite age of sale regulations

1.10 New NICE guideline

Developing the guideline

The National Institute for Health and Care Excellence (NICE) published its new guideline ‘Tobacco: preventing uptake, promoting quitting and treating dependence’ on 30 November 2021 (16). The extensive guideline brings together and updates all NICE's previous guidelines on using tobacco and presents recommendations on preventing uptake of smoking, promoting quitting, treating tobacco dependence and policy, commissioning and training.

The NICE committee carried out a number of systematic reviews covering the range of issues in its enhanced remit and included evidence on vaping products in a number of these. For example (and relevant to our evidence update), they reviewed the evidence on long-term health effects of vaping products, as part of the development stage of the new guidance (38).

The review aimed "to determine whether e-cigarettes cause any health harms or benefits aside from their potential to reduce smoking-related harm". It did not include "the potential reduction of harm of e-cigarettes when compared with smoking" but did include "the potential harms and benefits inherent to e-cigarette use alone".

The committee searched several databases and websites up until July 2020, using a broad approach to identify studies published since 1998, and followed up participants for a minimum of one year: They screened 6,907 non-duplicate articles, retrieved 118 articles for full text screening and included 2 articles in the review (39, 40).

The Bhatta and others study (40) is included in our new systematic review, but we excluded the Flacco and others study (39), because it only collected self-reported health outcomes. The NICE committee had serious concerns about these 2 studies and concluded they could not make recommendations about the long-term health effects of vaping as a result of the review.

Recommendations for smoking cessation and preventing tobacco use

NICE made 2 recommendations for preventing uptake of tobacco use:

> "As part of the curriculum on tobacco, alcohol and drug misuse, discourage children, young people and young adults who do not smoke from experimenting with or regularly using e-cigarettes. Talk about e-cigarettes separately from tobacco products.
>
> "When discussing e-cigarettes, make it clear why children, young people and young adults who do not smoke should avoid e-cigarettes to avoid inadvertently making them desirable."

For smoking cessation, NICE recommended that nicotine-containing vaping products should be accessible for adults who are trying to quit smoking, along with the existing range of support options. NICE's recommendations about advice that should be given about the use of vaping products for smoking cessation are set out below.

Advice on nicotine containing e-cigarettes

"Give clear, consistent and up-to-date information about nicotine-containing e-cigarettes to adults who are interested in using them to stop smoking.

Advise adults how to use nicotine-containing e-cigarettes. This includes explaining that:

- e-cigarettes are not licensed medicines but are regulated by the Tobacco and Related Products Regulations (2016)
- there is not enough evidence to know whether there are long-term harms from e-cigarette use
- use of e-cigarettes is likely to be substantially less harmful than smoking
- any smoking is harmful, so people using e-cigarettes should stop smoking tobacco completely

Discuss:

- how long the person intends to use nicotine-containing e-cigarettes for
- using them for long enough to prevent a return to smoking
- how to stop using them when they are ready to do so

Ask adults using nicotine-containing e-cigarettes about any side effects or safety concerns that they may experience. Report these to the MHRA Yellow Card scheme, and let people know they can report side effects directly.

Explain to adults who choose to use nicotine-containing e-cigarettes the importance of getting enough nicotine to overcome withdrawal symptoms, and explain how to get enough nicotine."

NICE also included a number of recommendations for research on vaping products in the guideline.

1.11 Selected international developments

Global policy scan

The Institute for Global Tobacco Control at Johns Hopkins University, Baltimore, US has continued its scan of country laws regulating vaping products. As of November 2021, they had identified 109 countries or other jurisdictions with regulations which they report in several policy domains (41).

Sale of vaping products was regulated in 85 countries, and 28 of those ban the sale of all types. Recent changes include Cambodia which banned the use, import and sale of vaping products in March 2021 (42) and Panama, where in July 2021, the National Assembly passed a bill banning vaping product imports, sales and use which has yet to be implemented (43). A minimum age of sale was identified in 56 countries or jurisdictions. Marketing was regulated or prohibited in 78 countries or jurisdictions with 6 of those only regulating marketing of nicotine-containing vaping products. Thirty-eight countries/jurisdictions had child safety packaging regulations and 51 required health warnings on packaging. Product regulations included 39 countries/jurisdictions which regulated nicotine concentration/volume, 39 that prohibited use of harmful ingredients (except nicotine) in e-liquid or regulated flavours in e-liquid and 34 that regulated quality of e-liquid content, required safety and quality evaluation, or had instituted other safety-related regulations for vaping products. Pre-marketing notification and additional reporting requirements, such as annual report of vaping product sales was required in 42 countries/jurisdictions. Sixty-six countries/jurisdictions prohibited or restricted vaping in public places; this included 13 that banned vaping completely. Finally, 33 countries/jurisdictions were identified that taxed vaping products.

World Health Organization

In July 2021, the WHO published a report on the global tobacco epidemic (44) which focused on addressing new and emerging products, which mostly meant vaping products (despite those not containing tobacco) including those without nicotine. Funded by Bloomberg Philanthropies, it emphasised harms of vaping to users, non-users, youth and tobacco control and stated that evidence on their potential role in smoking cessation was inconclusive. On that basis, the report recommended that ‘where ENDS are not banned, they should be regulated’ (44).

Recommendations included treating vaping as smoking in smoke-free places, not to use vaping for smoking cessation, strong graphic health warnings, bans on advertising, promotion and sponsorship, taxation as tobacco products, bans of online sales and sales to minors and consideration of bans on flavours. The report also contained a chapter on industry interference with a focus on ‘nicotine industry’. Both in this and a second report on tobacco regulation (45), the WHO called for a ban on devices which allowed users to control device features or liquid ingredients, which would mean that only disposable vaping products and cartridge or pod vaping products remained on the market. Given the data we show in chapter 4 on adult vaping, such a ban would seriously restrict what vaping products are currently being used by adults in England, and based on the exploratory research described earlier (10), could benefit the tobacco industry.

Call for a balanced policy

In September 2021, 15 US and UK scientists, all of them past presidents of the Society for Research on Nicotine and Tobacco (SRNT), published an article calling for a different balance in the consideration of the risks and benefits of vaping (46). After summarising health risks, main concerns around youth vaping and the role of vaping in increasing smoking cessation, they stated that the potential lifesaving benefits of e-cigarettes for adult smokers deserve attention equal to the risks to youths and concluded that vaping could have a much larger positive public health impact if more attention was paid to adult smokers. Several responses to the article from other scientists were published (47, 48), with one of the main points of criticism the original paper’s comments on a split of the field into opponents and supporters of vaping.

European Union

In April 2021, the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER), following a request from the European Commission, published their opinion on vaping products (49). This was to assist the Commission with its reporting obligations under Article 28 of the EU TPD and assist in assessing the need for any changes. SCHEER published the following conclusions in its abstract:

“The SCHEER concludes that on health effects

a) For users of electronic cigarettes

1. The overall weight of evidence is moderate for risks of local irritative damage to the respiratory tract of users of electronic cigarette due to the cumulative exposure to polyols, aldehydes and nicotine. However, the overall reported incidence is low.

2. The overall weight of evidence for risks of long-term systemic effects on the cardiovascular system is moderate.

3. The overall weight of evidence for risks of carcinogenicity of the respiratory tract due to long-term, cumulative exposure to nitrosamines and due to exposure to acetaldehyde and formaldehyde is weak to moderate. The weight of evidence for risks of adverse effects, specifically carcinogenicity, due to metals in aerosols is weak.

4. The overall weight of evidence for risks of other long-term adverse health effects, such as pulmonary disease CNS and reprotoxic effects based on the hazard identification and human evidence, is weak, and further consistent data are needed.

5. To date, there is no specific data that specific flavourings used in the EU pose health risks for electronic cigarette users following repeated exposure.

6. The overall weight of evidence for risks of poisoning and injuries due to burns and explosion, is strong. However, the incidence is low.

b) For secondhand exposed persons

1. The overall weight of evidence is moderate for risks of local irritative damage to the respiratory tract mainly due to exposure to glycols.

2. The overall weight of evidence for risks of systemic cardiovascular effects in second-hand exposed persons due to exposure to nicotine is weak to moderate.

3. The overall weight of evidence for carcinogenic risk due to cumulative exposure to nitrosamines is weak to moderate.

Electronic cigarettes are relatively new in terms of exposure to humans. More research is needed, in particular on long-term health effects.

Regarding the role of electronic cigarettes as a gateway to smoking/the initiation of smoking, particularly for young people, the SCHEER concludes that there is moderate evidence that electronic cigarettes are a gateway to smoking for young people. There is strong evidence that nicotine in e-liquids is implicated in the development of addiction and

that flavours have a relevant contribution for attractiveness of use of electronic cigarette and initiation.

Regarding the role of electronic cigarettes in cessation of traditional tobacco smoking, the SCHEER concludes that there is weak evidence for the support of electronic cigarettes' effectiveness in helping smokers to quit while the evidence on smoking reduction is assessed as weak to moderate."

SCHEER first published a preliminary opinion and opened it for consultation from 23 September to 26h October 2020 (50). They received 691 contributions and summarised that the most frequent comments related to the lack of comparison with tobacco smoking, the literature search and selection, the risk assessment methodology, the estimation of the risk of second-hand exposure, the delivery of nicotine by vaping products, the lack of recent data on vaping product use, and the conclusions on the gateway effect, attractiveness and cessation. SCHEER took into account some of the comments and included some of the suggested additional references (51). However, this did not address overall methodological weaknesses which we had identified in our 2021 report (5), namely that the methodology was not reported in sufficient detail in the report or annex to be able to understand how the evidence summarised had been selected. As we previously stated, established guidelines for systematically reviewing evidence and the reporting of reviews (52) had not been followed. For example, the quality of the studies included was not assessed and the search terms given for the review:

- did not capture all of the questions covered in the opinion
- had a start date of January 2015 and hence included studies of vaping products marketed long before the TPD was in place

Additionally, the report included predominantly studies from the US which therefore involved products which were regulated very differently from the TPD regulations.

United States

Postscript on ‘E-cigarette, or vaping, product use-associated lung injury' (EVALI) outbreak

The ‘EVALI’ outbreak was discussed in detail in our 2020 and 2021 reports (3, 5). The US Centers for Disease Control and Prevention (CDC) concluded that: ‘tetrahydrocannabinol (THC)-containing e-cigarette, or vaping products, particularly from informal sources like friends, family, or in-person or online dealers, are linked to most EVALI cases and play a major role in the outbreak” and “Vitamin E acetate is strongly linked to the EVALI outbreak’, and this was endorsed in a subsequent published paper (53). While the advent of vaping as a novel and less harmful drug delivery device provided the conditions for EVALI, it is now clear that EVALI was not caused by nicotine vaping. Unfortunately, as

discussed in chapter 15 (harm perceptions and communications), studies have shown that perceptions of the absolute harm, relative harm of vaping compared to smoking, and perceived addictiveness of vaping all increased after EVALI. This included one study carried out in the UK (54). An analysis of why nicotine vaping is not implicated in EVALI and the implications of the mislabelling and miscommunications around EVALI has been published, although not peer reviewed (55).

Authorisation process for vaping products

In the US, vaping product manufacturers were required to submit a Pre-Market Tobacco Product Application (PMTA) to the US Food and Drug Administration (FDA) by 9 September 2020 to receive approval to sell their products (56). This date was brought forward from August 2022 (57) following a court case brought by anti-tobacco groups (58).

There was a one-year period during which products with PMTA applications submitted were permitted to remain on the market pending the FDA review of the applications.

On 11 October 2021, the FDA gave its first marketing approval for a vaping product. Having previously denied applications from 55,000 flavoured tobacco products (59), Vuse Solo vaping closed device and tobacco-flavoured e-liquid pods (Vuse Solo Power Unit, and Vuse Replacement cartridges original 4.8% G1 and G2) gained clearance after the manufacturers (British American Tobacco's US subsidiary R. J. Reynolds Vapor Company) satisfied the FDA that the products could help smokers reduce exposure to harmful chemicals (60).

As we were finalising our report, further authorisations were issued to some LOGIC vaping products manufactured by Japan Tobacco International, additional VUSE products from R.J. Reynolds, and some NJOY vaping products manufactured by NJOY which is independent of the tobacco industry.

The US publishes figures for PMTAs at different stages of review but notes that the metrics can change due to the extremely large number of applications moving through the many steps of the review process, so it is stated that the data are generally accurate to within 10% (61). By 31 January 2022, PMTAs for over 8 million vaping products had been received, and just over a million marketing denial orders were given.

A spending bill passed in March 2022 by the US Congress that covered a wide range of topics also expanded the definition of an FDA-regulated 'tobacco product' to include those that use laboratory-made (synthetic) nicotine (62). Synthetic nicotine products now on the market have about 60 days from the signing of the law to submit an application to get a PMTA order and then the FDA has 90 days to issue an order. This may impact manufacturers and reduce the variety of brands on the market.

Canada

Building on a suite of regulations enacted by the federal government in Canada on vaping products and summarised in our previous reports (5, 7), in July 2021 Health Canada enacted regulations establishing a maximum nicotine concentration of 20mg/mL for vaping products (63, 64). Provincial, territorial and municipal laws also regulate vaping products and their use.

Australia

In Australia, it is illegal to import, possess or use nicotine liquid without a doctor's prescription. Until October 2021, consumers could import nicotine liquid for vaping from overseas for personal use through the Therapeutic Goods Administration (TGA) Personal Importation Scheme with a doctor's prescription. In October 2021, the Poisons Standard was amended to capture all nicotine vaping products as prescription-only medicines (65). The TGA said that the ban was designed to prevent young people taking up nicotine vaping.

Nicotine liquids remain available for purchase from some Australian pharmacies on prescription from a GP or the prescription can be used to import nicotine vaping products through the Personal Importation Scheme (66). A streamlined process for the writing and approval of nicotine prescriptions under the Authorised Prescriber Scheme was introduced along with training for doctors. Vape shops and other vendors are not able to sell or supply nicotine liquid.

The TGA has produced Questions and Answers for the change in regulations. There are currently no regulations for the safety and quality of nicotine liquid. However, child resistant closures were also made mandatory from 1 October 2021.

In February 2022, a consultation was opened (until 24 March 2022) on a new draft Australian National Tobacco Strategy 2022 to 2030 which set out a target of 10% daily smoking among adults by 2025 and 5% by 2030 (67). The existing National Tobacco Strategy 2012 to 2018 which also had a target of 10% daily smoking by 2018 remains in place until the new strategy is finalised. The new draft strategy sets out 11 priority areas. One priority area aims to strengthen regulations for novel and emerging products and includes an action to 'further restrict the marketing, availability and use of all e-cigarette components in Australia, regardless of their nicotine content'.

New Zealand/Aotearoa

In our last report (5) we described the Smoking Environments and Regulated Products (Vaping) Amendment Act 2020 (2020/62) which was passed on 11 August 2020 (68). The Act broadened the scope of products regulated under the Smoke-free Environments Act

1990 to include vaping products and heated tobacco products, with scope to add new regulated products if appropriate in the future. The Act acknowledged that vaping products and heated tobacco products had lower health risks than smoking and aimed to strike a balance between supporting smokers to switch to the less harmful products while improving their safety and limiting young people's access and attraction to them.

In our last report (5) we listed the provisions of the Amendment Act, some of which had come into force in November 2020. In 2021, additional provisions came into force (69) including the need for approval as Specialist Vape Retailers for retailers wishing to sell flavoured vaping products (other than tobacco, mint or menthol, which could still be sold by general retailers), the prohibition of colouring substances, and signage and notices for schools, general and Specialist Vape Retailers regarding vaping.

On 28 November, the law on smoking in cars (Prohibiting Smoking in Motor Vehicles Carrying Children) was amended and came into force making smoking and vaping in motor vehicles carrying under 18 year olds to be against the law.

In December 2021, following a consultation carried out in April and May of that year (70), the New Zealand government published its Smokefree Aotearoa, 2025 Action Plan (71). This set out a bold plan to reach a target of fewer than 5% daily smokers across all societal groups by 2025, acknowledging that based on current trends this would take decades to achieve.

Three outcomes were set out:

1. Eliminate inequities in smoking rates and smoking-related illnesses - this acknowledged the marked inequities in smoking prevalence and consequent health outcomes among Maori, Pacific people and those living in the most deprived areas of New Zealand.

2. Create a smokefree generation by increasing the number of children and young people who remain smokefree – this acknowledged the need to protect children from smoked tobacco products and second-hand smoke exposure including the need to ensure smoked tobacco products were neither appealing or addictive.

3. Increase the number of people who successfully quit smoking – this acknowledged the need to address the wide availability of smoked tobacco products particularly in disadvantaged neighbourhoods, and their addictive nature. It also recognised that support for stopping smoking or for switching to less harmful alternatives needed to be made available.

Six focus areas were set out and of relevance to this report we highlight actions relevant to or likely to impact vaping:

1. Ensure Maori leadership and decision-making at all levels.

2. Increase health promotion and community mobilisation (including funding a health promotion programme to prevent young people from vaping).

3. Increase evidence-based stop smoking services.

4. Reduce the addictiveness and appeal of smoked tobacco products (including allowing only very low nicotine levels in smoked tobacco products).

5. Reduce the availability of smoked tobacco products (including introducing an authorisation scheme for retailers to sell tobacco products, and prohibiting the sale of smoked tobacco products to persons born after a certain date, and introducing a notification scheme for general retailers to advise the Director-General of Health before selling vaping products).

6. Ensure manufacturers, importers and retailers meet their legal obligations (in relation to sale and supply of smoked tobacco products within New Zealand).

By February 2022, all manufacturers and importers of vaping products were required to have notified the Vaping Regulatory Authority about the products they intended to sell in New Zealand after that date and the products also needed to meet safety requirements (72). Notifications must be renewed annually or the notifications expire. If a product undergoes a significant change post-notification, a new notification needs to be completed and the existing one cancelled.

Further provisions of the Amendment Act will be introduced later this year in relation to packaging requirements and their distribution and sale and in 2023 a requirement for manufacturers, importers and Specialist Vape Retailers to submit annual reports and returns for the previous year.

1.12 Scope of this report

Due to ongoing reviews, this report does not cover the question of whether vaping acts increases the risk of subsequent smoking in people who would otherwise not have smoked (gateway hypothesis) which is covered by a forthcoming, comprehensive Cochrane Review (73). The report also does not cover the effectiveness of vaping products for smoking cessation which is addressed by an ongoing living Cochrane Review (74). COVID-19 continues to affect the implementation of routine surveys which we use in chapter 3 (vaping among young people) and chapter 4 (vaping among adults). COVID-19 also undoubtedly has affected both vaping and smoking behaviours in England but a full assessment of this is outside the scope of this report.

1.13 Main findings

As in our 2020 and 2021 reports (3, 5), we use the term ‘vaping products’ to describe e-cigarettes and refill containers (e-liquids) intended for nicotine vaping. Some vaping products do not always contain nicotine. Where studies explored products without nicotine, we refer to them as non-nicotine vaping or vaping products. We use ‘vapers’ to refer to people who regularly use vaping products and ‘vaping’ as the act of using a vaping product. These terms do not include cannabis vaping or the vaping of other legal or illegal substances, which are not the subject of this report.

Under the TRPR, vaping products need to be notified to the Medicines and Healthcare products Regulatory Agency (MHRA) before they can be legally sold in the UK. An analysis of 2016 to 2017 notifications found that notified products were unlikely to cause serious harm. However, reporting and surveillance should be standardised to maximise any harm reduction potential.

Non-nicotine containing vaping products come under the General Product Safety Regulations 2005, enforced by local authority trading standards.

Medicinally licensed vaping products are exempt from the TRPR and currently there is no licensed product in the UK, although updated guidance was published by the MHRA in October 2021 to provide greater clarity on the process and expedite review times.

The MHRA also collects information on adverse events believed to be associated with nicotine containing vaping products through its Yellow Card scheme. Between 20 May 2016 (implementation of TRPR) and 13 January 2022, the MHRA had received 257 reports of adverse reactions (26 of those since January 2021). Each report represents an individual for whom more than one adverse reaction could have been reported. A report is not proof that the reaction was caused by a vaping product, just that the reporter thought it might have been. Since January 2021, 14 of the reports were considered serious. There have been no fatalities. Adverse reactions are also reported for licensed smoking cessation medications (297 reports for NRT and 78 for varenicline in 2021). Varenicline has been unavailable since June 2021, further limiting effective pharmaceutical options for smoking cessation.

It is illegal to sell vaping products to anyone aged under 18 and to buy vaping products for anyone under 18. There is a loophole in the legislation allowing free samples of vaping products to be given to people of any age. Surveys carried out by the Chartered Trading Standards Institute to capture tobacco control activities including enforcement of age of sale vaping and tobacco product laws, have not been carried out since 2020. A specific project carried out in Scotland between October and December 2021 focused on single use disposable vaping products finding a majority had not been notified as required with many above the 20mg/mL nicotine content limit. Some violations of age of sale laws were also identified in Scotland. A review of the age of sale legislation published in January

2021 concluded that overall, it had achieved its original objective of reducing uptake among under 18s.

A government consultation in 2019 outlined a new ambition to go smokefree in England by 2030 (defined as adult smoking prevalence of 5% or less). It included an ultimatum to industry to make smoked tobacco obsolete by 2030, with smokers quitting or moving to reduced risk nicotine delivery systems, such as vaping products.

The All-Party Parliamentary Group on Smoking and Health made recommendations to help achieve the smokefree 2030 ambition. These included reducing the appeal and availability of vaping products and other nicotine products to young people and adapting the route for medicinal licensing to allow vaping products to be authorised for prescription.

A review of vaping product marketing in the UK between 2016 and 2019 found high compliance with the advertising code in advertisements but non-adherence in social media posts. It found that young people who had never smoked or vaped noticed posts relating to vaping more than adults who smoked. Compared with the US and Canada however, UK regulations were found to have limited exposure to marketing among adults and youth.

The UK government published its review of the Tobacco and Related Products Regulations 2016 (TRPR), which govern nicotine vaping products, in March 2022 to assess whether the regulations have met their objectives. This post implementation review concluded that the evidence presented indicated the main objectives were being met, providing a strong argument for the retention of the regulations. It also proposed some amendments which could help support the government's smokefree 2030 ambition.

A new tobacco control plan for England was expected in July 2021 outlining the strategy to achieve the smokefree 2030 ambition but will now be published in 2022. The 2017 tobacco control plan for England, which sets out ambitions for 2022, remains in place, although progress towards meeting the ambitions has been mixed.

An independent review of tobacco control, which was published in June 2022 was commissioned by the government to identify the best ways to address health inequalities caused by smoking and to achieve the smokefree 2030 ambition.

Vaping products which do not contain nicotine and are regulated through the General Product Safety Regulations 2005 are less strictly regulated than nicotine-containing products. So, their regulation requires further consideration. As other non-tobacco nicotine products (such as nicotine pouches) emerge in the UK, it seems appropriate to review regulations for these products at the same time.

In November 2021, NICE published a new comprehensive guideline on tobacco including guidance on preventing uptake of smoking, promoting quitting, treating tobacco dependence and recommendations for policy, commissioning and training. This includes

guidance on discussing vaping products in the context of preventing tobacco use and detailed guidance on discussing vaping products for smoking cessation.

We also summarise recent international developments in vaping product policy, including in the European Union, the United States, Canada, Australia and New Zealand/Aotearoa. In New Zealand a bold strategy was launched in December 2021 to reduce daily smoking to less than 5 per cent for all groups by 2025, and the strategy included provisions to encourage stopping smoking as well as switching to alternative nicotine products such as vaping products.

1.14 Implications

The smokefree 2030 ambition and developing a new tobacco control plan for England provide an opportunity to review all vaping (and other nicotine and tobacco) regulations to ensure that they are appropriate and help smokers quit, while managing the risk of uptake for never smokers.

The next tobacco control plan provides an opportunity to set out the bold plans needed to reach the smokefree 2030 ambition for all groups and to set intermediate targets for smoking prevalence in different disadvantaged groups.

The continuing lack of a medicinally licensed product is of concern and may require further review of the process involved.

Further attention is needed as to whether some aspects of packaging of vaping products need restricting.

Substantially strengthened enforcement of marketing regulations on social media is indicated.

There is an opportunity for the notification processes to be standardised to enable research using the MHRA database of notified vaping products and to maximise harm reduction potential.

Local authority trading standards efforts have been scaled down and compliance with regulations is inadequate to prevent underage sales and access to illicit products. Also, more frequent surveillance of single-use disposable vaping products is needed. There is a danger that the reduction in local trading standards officers and restructure of the MHRA could result in a lack of surveillance of these products which could undermine the approach and regulatory framework for vaping products adopted in England.

Lessons should be learned from the mislabelled US e-cigarette or vaping use-associated lung injury (EVALI) outbreak. These lessons include the impact of miscommunications

about nicotine vaping compared to vaping contaminated illicit substances. Communications about EVALI should clearly separate vaping these illicit substances from nicotine vaping. Also, communications about any future cases or outbreaks of poisonings or injuries should be clear about the implicated substances.

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2 Methods

2.1 Methods: vaping among young people and adults

This chapter describes the methods used for the chapters on vaping among young people (chapter 3) and vaping among adults (chapter 4). As with previous Vaping in England reports (1-5), this one also used several survey data sources to explore vaping characteristics among young people and adults in England.

Survey data used

To assess vaping and smoking among young people in England, we used data from the Action on Smoking and Health Smoke-free Great Britain Youth survey (ASH-Y) and the International Tobacco Control Policy Evaluation Project Youth Tobacco and Vaping Survey (ITC Youth). The methods, sampling strategies and sample size of these 2 surveys are described in table 1 and data reported in chapter 3.

To describe smoking and vaping among adults in England, we used the Annual Population Survey (APS), the annual ASH Smoke-free Great Britain Adult Survey (ASH-A), the Smoking Toolkit Study (STS) data and the Opinions and Lifestyle Survey (OPN). Characteristics of these surveys are described in table 2 and data reported in chapter 4.

In May 2022 as we were finalising our report, we became aware that ASH was to publish reports from their 2022 ASH-Y and 2022 ASH-A survey findings around the same time as we would be publishing our report. For consistency across our report and the ASH reports, we therefore incorporated top-line smoking and vaping prevalence data from the 2022 ASH-Y and ASH-A surveys (collected February to March 2022) into chapter 3 and chapter 4. As the 2022 ASH-Y and ASH-A survey data also identified a change in the types of vaping products used, and in line with issues identified by trading standards officers about disposable vaping products discussed in our Introduction chapter, we also included the 2022 ASH-Y and ASH-A data on types of vaping products used. Given time constraints, we were unable to include other 2022 ASH-A and ASH-Y data but the full report from the 2022 ASH-Y and ASH-A surveys survey will be available on the ASH website. Please note that the findings will not exactly match as our report focuses on data from England, whereas the ASH surveys cover Great Britain. Also, the ASH-Y report focuses on 11 to 17 year olds whereas we mainly cover data from 11 to 18 year olds.

The coronavirus (COVID-19) pandemic affected data collection for several surveys. The APS survey mode changed from predominantly face to face in January to March 2020 to telephone only interviews in April to December 2020, which has impacted estimates of the proportion of adults who smoke cigarettes (6). We provide both estimates—before and after the change in data collection modality. Following the interruption in data collection for

the STS survey in March 2020, data for the subsequent months were collected using computer-assisted telephone interviewing rather than household surveys. Because of social distancing restrictions under the COVID-19 lockdown, STS data from April 2020 onwards were collected by telephone interviews and only from people aged 18 and over (rather than 16 and over as in previous face-to-face interviews). We adjusted STS data comparisons over time in the last report (1) and continue to analyse only data of participants aged 18 years old or older. Comparison of the STS data before and after changing the data collection method suggests no change in data quality and allows for comparisons before and after the first lockdown in March 2020 (7, 8).

Table 1. Surveys used, young people

Survey name and acronym	Commissioned and conducted by	Geographic coverage, sample	Age	Representativeness	Design or mode
ASH-Y Action on Smoking and Health (ASH) Smoke-free Great Britain Youth survey	ASH and YouGov Plc	Annual GB survey of ~2,500 young people. In 2021 the survey conducted in March to April; GB n = 2,513, England n = 2,151. In 2022 the survey conducted between February and March; GB n = 2,613, England n = 2,259. Recruited from a YouGov Plc UK panel of more than 800,000 members	11 to 18 years	Figures weighted to be representative of GB youth aged 11 to 18	Online, repeated, cross-sectional survey
ITC Youth International Tobacco Control Policy Evaluation Project (ITC) Youth Tobacco and Vaping survey	School of Public Health Sciences, University of Waterloo and the Nielsen Consumer Insights Global Panel	England sample of ~4,500 young people. Survey (Wave 4.5) conducted in February 2021. England n = 4,224. We also use data from surveys carried out in August to September 2019 and February 2020.	16 to 19 years	Data are weighted to be representative of demographic characteristics (age by sex by region), calibrated to wave 1 sample proportions for student status and school grades, and to past 30-day smoking trend (in Canada and the US only)	Online, repeated, cross-sectional survey. Data collection is carried out using non-probability sampling

Notes: for both surveys, participants from England only were used in the analyses presented in this report.

More information on the ITC Youth surveys can be found on the ITC Youth Tobacco and Vaping Survey website.

Table 2. Surveys used, adults

Survey name and acronym	Commissioned and conducted by	Geographic coverage, sample and date of most recent survey	Age	Representativeness	Design or mode
APS Annual Population Survey	The Office for National Statistics (ONS)	UK survey of 44,327 respondents collected by mixed household and phone interviews in January to March 2020, and of 121,346 respondents collected by phone in April to December 2020. England January to March 2020 n = 31,265; England April to December 2020 n = 88,897.	18+ years	Systematic sampling ensures representativeness at a regional level. Weighting is used to reflect official UK population data	Annual household survey conducted face-to-face or by telephone until March 2020. Since April 2020, data collection changed to telephone only interviews.
STS Smoking Toolkit Study	University College London and Ipsos MORI	England survey of ~1,700 people per month, England 2021 (to September) n = 14,758	18+ years	The sample is weighted to be representative of census data for adults in England	Data collected using computer-assisted telephone interviewing

Survey name and acronym	Commissioned and conducted by	Geographic coverage, sample and date of most recent survey	Age	Representativeness	Design or mode
ASH-A ASH Smoke-free Great Britain Adult Survey	ASH and YouGov Plc	Annual GB survey, in 2021 conducted between February and March, GB n = 12,247, England n = 10,211. In 2022 conducted between February and March, GB n = 13,088, England n = 10,883. Recruited from a YouGov Plc UK panel of more than 800,000 members	18+ years	The data are weighted to be representative of GB adults	Online, repeated, cross-sectional survey
OPN Opinions and Lifestyle Survey	The Office for National Statistics (ONS)	GB survey of people with a history of smoking and/or vaping, n = 71.286.	16+ years	Recruited from a panel using quotas for age, gender and region	Fortnightly (weekly between March 2020 and August 2021) cross-sectional sample survey by predominantly online (telephone interviews available if requested by a respondent) data collection.

Notes: for all surveys, participants from England only were used in the analyses presented in this report.

NHS Digital stop smoking service data

Data are collected by NHS Digital from local authority commissioned services every 3 months about: the number of quit attempts made (people can make several quit attempts in one year and therefore be counted more than once); the number of quit attempts which led to successful quits at 4 weeks (self-reported and carbon-monoxide (CO) verified); and key measures of the service including intervention type, intervention setting and type of pharmacotherapy received. Since 2014, stop smoking services have been asked to record if a vaping product, alone or in combination with other smoking cessation aids, was used in a quit attempt.

A person is counted as a 'self-reported 4-week quitter' if they are assessed (face to face or by telephone) 4 weeks after the designated quit date and declare that they have not smoked a single puff on a cigarette in the past 2 weeks. A person is counted as a CO-verified 4-week quitter if they are a self-reported 4-week quitter and their expired-air CO 4 weeks after their designated quit date (-3 or +14 days) is less than 10 parts per million (9, 10). People who have set a quit date and are lost to follow up are counted as nonquitters. However, CO monitoring has been disrupted recently given COVID-19 related restrictions.

NHS Digital does not provide information on statistical difference and we do not have access to the raw data to provide more detailed information. In chapter 4, we report more detail on NHS Digital methods and stop smoking service data from April 2020 to March 2021.

Statistical testing

In this report, where it was feasible, we introduced testing for statistically significant differences between comparison groups. Due to large enough sample sizes, we tested differences between groups in population-representative adult samples using STS and ASH-A data (chapter 3).

To test for relationships between 2 categorical variables, we used Pearson's chi-squared (χ^2) test of independence. For the statistical testing, unweighted counts were used, and groups that did not comprise more than 50 participants were not included in the testing. We also highlighted instances where cells with expected count less than 5 were included in the testing to indicate that the outcome of these tests might not be statistically reliable and should be interpreted with care. A p-value less than 0.05 was considered to indicate a statistically significant difference between groups.

2.2 Methods: systematic literature review of the health risks of vaping

The following paragraphs describe the methods used for a systematic literature review of the health risks and health effects of vaping. The protocol of this review was registered on PROSPERO, the international prospective register of systematic reviews.

Review questions

1. What effect does vaping and secondhand exposure to vaping products have on biomarkers that are associated with the risk of cancer, respiratory, cardiovascular or other health conditions?

2. What are the effects of vaping among people with existing health conditions (as above) on disease outcomes?

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCT), non-randomised studies, cross-over studies, single group studies, longitudinal and cross-sectional studies. As a secondary outcome, we aimed to review how vaping is associated with poisonings, fires and explosions (Chapter 13), therefore we also included case and case series studies for this outcome. We included all peer-reviewed papers—both published and in press. Publications in English, French and German were considered for the review.

We excluded qualitative studies and non-peer-reviewed literature (for example, research posters, conference abstracts, PhD theses, research letters).

Condition of domain being studied

We summarised studies reporting on firsthand and secondhand exposure to nicotine vaping in humans, animals and cells. Nicotine vaping products comprise of a battery-powered heating element designed to vaporise a solution made of propylene glycol and/or glycerine, water and usually flavouring compounds, flavour enhancers, and nicotine (though some vaping products do not always contain nicotine); the vapour (aerosol) resulting from nicotine vaping devices is then inhaled. Vaping cannabis or other legal or illegal substances was not the subject of the systematic review.

Types of participants

We included studies reporting on human participants (youth and adults) exposed to vapour through direct use of or secondhand exposure to vaping products.

We recognise that substantial proportions of people who vape are ‘dual users’ who vape and smoke. The heterogeneity of dual users has been demonstrated elsewhere including in studies in which we were involved, for example, in Borland and others (11) we recommended a 4-level typology for characterising concurrent users based on frequency of use, and the different dual use groups varied across level of dependence, attitudes and intentions. Unfortunately, in the vast majority of our included studies, dual or concurrent users were not well defined or put into meaningful categories. Given the scope of our work, we decided that our prime focus would be comparisons between exclusive vapers and smokers, and exclusive vapers and non-users. Data on concurrent use were extracted where feasible and provided in summary tables.

We also included studies reporting on animals, human and animal cells exposed to aerosol produced by vaping products.

Types of interventions

Firsthand or secondhand exposure to vaping products with or without nicotine over any time frame. For longitudinal human studies, we categorised exposure times as:

1. Acute exposure: from single use to 7 days.

2. Short- to medium-term exposure: from 8 days to 12 months.

3. Long-term exposure: more than 12 months.

Types of outcome measures

Main outcomes

Biomarkers of nicotine and potential toxicant exposure: we summarised studies reporting on the levels of biomarkers in participants’ biosamples (for example, blood, urine, saliva, hair) after vaping or secondhand exposure to vapour from nicotine vaping products. We extracted data on priority toxic contents and emissions of tobacco products reported by the World Health Organization (WHO) (12), which included biomarkers for nicotine and its metabolites, carbon monoxide, tobacco specific nitrosamines, volatile organic compounds, polyaromatic hydrocarbons, aromatic amines, metals and other toxicants and carcinogens (table 3).

Biomarkers of potential harm to health: we summarised studies reporting on associations of vaping with objectively measured biomarkers of harm (surrogate endpoints) related to cancers, respiratory and cardiovascular health, oxidative stress, inflammation, endothelial function and other health markers (table 4). We did not extract subjective self-reported data that could be associated with potential harm to health. Biomarkers of interest were informed by findings from the US Food and Drug Administration sponsored workshop on biomarkers of potential harm associated with tobacco and nicotine products (13).

Table 3. Priority toxic contents and emissions of tobacco products reported by the WHO, their biomarkers and associated characteristics

Category	Toxicant	Toxicant characteristics	Metabolites	Metabolite characteristics
Nicotine	Nicotine	FDA[a]: RDT, AD Also considered as a cardiovascular toxicant (14).	Nicotine	Blood and urinary nicotine levels serve as its own exposure indicator. $t_{1/2}$: 1-2 hours (15).
			Cotinine	$t_{1/2}$: 16-18 hours (15). Primary metabolite of nicotine
			Total nicotine equivalents (TNEQ)	Sum of nicotine, cotinine and trans-3-hydroxycotinine. Accounts for >90% of the nicotine dose therefore reflects nicotine exposure and is not affected by individual metabolic differences.
			trans-3'-hydroxycotinine (3HC)	$t_{1/2}$: 4.6 hours (15). Made during the hydrolysis of cotinine process. The ratio of cotinine/3HC reflect the rate of metabolism of nicotine.
Volatile organic compounds	Acetaldehyde	FDA: CA, RT, AD IARC[b]: group 2B carcinogen	Acetate	$t_{1/2}$: 18-31 minutes (16).

Category	Toxicant	Toxicant characteristics	Metabolites	Metabolite characteristics
	Acrolein	IARC: group 2A carcinogen Also has cardiovascular effects (17).	3-HPMA	$t_{1/2}$: 9 hours (18).
			CEMA	$t_{1/2}$: 8 hours (19).
	Acrylamide	FDA: CA IARC: group 2A carcinogen	AAMA	$t_{1/2}$: 11-17.4 hours (15), 14 hours (18).
			GAMA	$t_{1/2}$: 19-25.1 hours (15).
	Acrylonitrile	FDA: CA, RT IARC: group 2B carcinogen	CNEMA	$t_{1/2}$: 8 hours (18).
	Benzene	FDA: CA, CT, RDT IARC: group 1 carcinogen	S-PMA	$t_{1/2}$: 9 hours (15, 18).
			MU	
	1,3-Butadiene	FDA: CA, RT, RDT IARC: group 1 carcinogen	DHBMA; MHBMA3	$t_{1/2}$: 5-9 hours (20).
	Butyraldehyde	Has effects on respiratory health.		
	Crotonaldehyde	FDA: CA IARC: group 2B carcinogen Causes oxidative stress.	CMEMA; HMPMA	$t_{1/2}$: 5-9 hours (20).
	Formaldehyde	FDA: CA, RT IARC: group 1 carcinogen	Formate	$t_{1/2}$: 2.5-12.5 hours (21). Formate levels vary considerable across humans.
	Hydroquinone	IARC: group 3 carcinogen	1,4-benzoquinone	$t_{1/2}$: 15 hours (22). Hydroquinone is a metabolite of benzene.
	Isoprene	FDA: CA IARC: group 2B carcinogen	IPM1; IPM3	$t_{1/2}$: 85 minutes in rodents (23).
	Propionaldehyde	FDA: RT, CT IARC: group 3 carcinogen	Propionic acid	

Category	Toxicant	Toxicant characteristics	Metabolites	Metabolite characteristics
	Toluene	FDA: RT, RDT IARC: group 3 carcinogen Respiratory toxicant and has effects on central nervous system.	S-BMA	$t_{1/2}$: <10 hours (15), 12.9 hours (24)
Tobacco-specific nitrosamines	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	FDA: CA IARC: group 1 carcinogen NNK and NNAL induce tumours in laboratory animals and increase the lung cancer risk in smokers (25, 26)	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	$t_{1/2}$: 10.3 days (15).
	Anabasine	IARC: group 3 carcinogen	N-Nitrosoanabasine (NAB)	$t_{1/2}$: 30 minutes in rabbits (27)
	Anatabine	IARC: group 3 carcinogen	N-Nitrosoanatabine (NAT)	$t_{1/2}$: 90 minutes in rabbits (27)
	Nornicotine	FDA: CA IARC: group 1 carcinogen Induces tumours in laboratory animals and cancer of the oral cavity and oesophagus in humans (25, 26).	N-Nitrosonornicotine (NNN)	$t_{1/2}$: 45 minutes in rhesus monkeys (28)
Polyaromatic hydrocarbons	Benzo[a]pyrene	FDA: CA IARC: group 1 carcinogen	3-hydroxy-benzo[a]pyrene (Total-3-OHB[a]P)	$t_{1/2}$: 12-18 hours (29)
	Pyrene	IARC: group 3 carcinogen	1-HOP	$t_{1/2}$: 18-20 hours (30)

Category	Toxicant	Toxicant characteristics	Metabolites	Metabolite characteristics
Aromatic amines	1-Aminonaphthalene (1-Naphthylamine)	FDA: CA IARC: group 3 carcinogen	1-AN	
	2-Aminonaphthalene (2-Naphthylamine)	FDA: CA IARC: group 1 carcinogen	2-AN	
	3-Aminobiphenyl		3-ABP	
	4-Aminobiphenyl	FDA: CA IARC: group 1 carcinogen	4-ABP	$t_{1/2}$: 15.6 hours in rodents (31).
Other	Acetone	FDA: RT		$t_{1/2}$: 17-27 hours (32).
	Ammonia	FDA: RT		$t_{1/2}$: <3 minutes
	m-Cresol	FDA: CA, RT A cardiovascular toxicant (33).		
	p-Cresol	FDA: CA, RT		$t_{1/2}$: 1.5 hours in rodents (34)
	o-Cresol	FDA: CA, RT		
	o-Toluidine	FDA: CA IARC: group 1 carcinogen	o-Toluidine (o-Tol)	$t_{1/2}$: <48 hours (35).
	Catechol	FDA: CA IARC: group 2B carcinogen		$t_{1/2}$: 3-7 hours. Catechol is a metabolite of benzene.
	Hydrogen cyanide	FDA: RT, CT Also has neurological and thyroid effects.	Thiocyanate	$t_{1/2}$: 1-2 weeks (36)
	Nitric oxides	Respiratory toxicant (37).	Exhaled breath nitric oxide (eNO)	$t_{1/2}$: 0.05-1 second in blood (38)
	Phenol	FDA: RT, CT IARC: group 3 carcinogen		$t_{1/2}$: 16.3 hours (22). Phenol is a metabolite of benzene.

Category	Toxicant	Toxicant characteristics	Metabolites	Metabolite characteristics
	Pyridine	IARC: group 2B carcinogen Potential reproductive or developmental toxicant.	3-hydroxypyridine	
	Resorcinol	IARC: group 3 carcinogen Has effects on central nervous system (39).		$t_{1/2}$: 31 hours (39).
	Quinoline	IARC: group 2B carcinogen	3-hydroxyquinoline	
Carbon monoxide	Carbon monoxide	FDA: RDT Contributes to the increased risk of non-fatal and fatal myocardial infarction and sudden death from coronary heart disease (40).	Expired air CO (eCO)	$t_{1/2}$: < 5 hours (41)
			Carboxyhaemoglobin (COHb)	$t_{1/2}$: 5 hours (41)
Metals	Arsenic	FDA: CA, CT, RDT IARC: group 1 carcinogen Exposure to arsenic is associated with an increased risk of cardiovascular disease (42).		$t_{1/2}$: 10 hours (43)
	Cadmium	FDA: CA, CT, RDT IARC: group 1 carcinogen Also has respiratory effects and cardiovascular effects (42).		$t_{1/2}$: 13.6 years (15).

Category	Toxicant	Toxicant characteristics	Metabolites	Metabolite characteristics
	Lead	FDA: CA, CT, RDT IARC: group 2B carcinogen Exposure to lead is associated with an increased risk of cardiovascular disease (42).		$t_{1/2}$: 1-2 months in blood and soft tissues years in bones (15).
	Mercury	FDA: CA, RDT IARC: group 3 carcinogen	Methylmercury	$t_{1/2}$: 50-80 days (44)

Notes: [a] Potential effect on human health according to the FDA established list of harmful and potentially harmful constituents (HPHC) in tobacco products (45). AD—addictive; CA—carcinogen; CT—cardiovascular toxicant; RDT—reproductive or developmental toxicant; RT—respiratory toxicant.

[b] Classification of a toxicant based on the International Agency for Research on Cancer (IARC) (46). Group definitions are: 1) Group 1: carcinogenic to humans; 2) Group 2A: probably carcinogenic to humans; 3) Group 2B: possibly carcinogenic to humans; 4) Group 3: not classifiable as to its carcinogenicity to humans.

Table 4. Biomarkers of potential harm and associated health risks

Category	Measures	Associated health risks	Other details
Respiratory outcomes	Forced expiratory volume (FEV1)	Respiratory	Surrogate endpoint for COPD (13). Lung function remains stable between 20 and 35 years of age, FEV1 then declines due to the natural aging processes, with an accelerated decline after 70 years of age (47). FEV1 decline slows down to normal after stopping smoking (13). Changes in FEV1 may require several years to detect (13).
	Forced vital capacity (FVC)	Respiratory	
	FEV1/FVC ratio	Respiratory	

Category	Measures	Associated health risks	Other details
	Fractional exhaled nitric oxide (FeNO)	Respiratory	Biomarker of asthma (47). Complete abstaining from smoking for 52 weeks leads to near normalization of FeNO levels (48).
	Peak expiratory flow (PEF)	Respiratory	
Cardiovascular outcomes	Heart rate	CVD	Higher resting heart rates have been associated with premature mortality and stroke (49). After adjusting for physical fitness, an independent risk factor for all-cause mortality in men but not women (50).
	Systolic blood pressure	CVD	A well-established risk factor for all-cause mortality, stroke, CVD. Systolic blood pressure increases with age. Systolic blood pressure is a function of increasing vascular stiffness and endothelial dysfunction.
	Diastolic blood pressure	CVD	The significance of borderline-moderately high diastolic blood pressure in the absence of systolic hypertension (isolated diastolic hypertension) remains unknown.
	Blood oxygen saturation	CVD	
	Heart rate variability	CVD	Biomarker for incident stroke and post-stroke outcomes (51).
	Coronary artery calcification (CAC) score	CVD	Biomarker of subclinical atherosclerosis (13). Predictive of coronary heart disease (13). A marker of an advanced stage of the disease process, may not be reversible (13). An independent predictor of CV risk in low risk/asymptomatic patients.

Category	Measures	Associated health risks	Other details
	Arterial stiffness (pulse wave velocity)	CVD	Biomarker of central aortic stiffening (52). Associated with higher risk of cardiovascular events (52). There is limited evidence indicating that e-cigarettes alter arterial stiffness (52).
Oxidative stress markers			A disturbance in the balance between production of reactive oxygen species and antioxidant defences. Associated with damage and impaired function of lipids, proteins, and DNA in ways that impair cellular function (13)
	F2 isoprostane	CVD	Formed in vivo through free radical-induced peroxidation of arachidonic acid. Relationship with cancer risk is not yet established (13). Levels reduced when smokers switch to other tobacco products (13).
	Oxidized low-density lipoprotein (LDL)	CVD	Lipoprotein levels in blood are thought to be causally related to CVD. Tobacco smoking is related with an increase of LDL levels (52). Transport cholesterol and can be involved with the development of atherosclerotic plaque (52). Non-smoker levels might be reached about 10 months after stopping smoking (53). LDL levels are highly related to diet, physical activity and genetics.

Category	Measures	Associated health risks	Other details
	High-density lipoprotein (HDL)	CVD	Lipoprotein levels in blood are thought to be causally related to CVD. Tobacco smoking is related with a decrease of HDL levels (52). Transport cholesterol away from cells, with levels inversely related to CVD and smoking. Non-smoker levels might be reached about 3 months after stopping smoking (53). HDL levels are highly related to diet, physical activity and genetics.
	Triglycerides (TGs)	CVD	Main constituent of fat cells and enable transportation of adipose fat and blood glucose from the liver. High levels of triglycerides in the bloodstream have been linked to atherosclerosis, heart disease and stroke (52). TGs levels are highly variable and relate to diet and the time of blood collection; levels after fasting are most valid.
	8-hydroxy-2'-deoxyguanosine (8OHdG)	Cancer, CVD	A product of DNA oxidation damage caused by oxidative stress/oxygen-free radicals (54). The adduct is formed from reactive oxygen species physiologically formed from oxygen. Estimated from the urinary excretion of 8OHdG, tobacco smoking was reported to increase the oxidative DNA damage by 30%-50%. However, traffic emissions might induce a higher level of oxidative stress towards DNA damage measured by this biomarker than smoking (53).

Category	Measures	Associated health risks	Other details
	8-isoprostane (8-iso-prostaglandin F2α)	Cancer, CVD, Respiratory	A marker of antioxidant deficiency and oxidative stress, a type of F2 isoprostane. Tobacco smoking caused a relatively small increase in free 8-iso-PGF2a (55). There is no substantial evidence of a strong direct link between 8-iso-PGF2a and smoking cessation except after long periods of time (47). The effects of e-cigarettes were less pronounced than those caused by traditional tobacco cigarettes, especially regarding the levels of 8-isoPGF2a (56).
	Serum levels of vitamin C	CVD	Increasing plasma ascorbic acid concentration was strongly and independently associated with reduction in risk of mortality from all causes, cardiovascular disease, and ischaemic heart disease, with a dose-response relation (57). Diet and use of dietary supplements might significantly affect the serum levels of vitamin C. Supplementation of Vitamins ACE have failed to reduce CV events in numerous clinical trials (Physicians Health Study II).
	Reactive oxygen species (ROS)	Cancer	Excess of ROS can damage cellular lipids, proteins, or DNA, thus inhibiting signal transduction pathways and normal cellular functions.

Category	Measures	Associated health risks	Other details
	Soluble NOX2-derived peptide (sNOX2-dp)	CVD	A marker of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase activation. The effects of e-cigarettes were less pronounced than those caused by traditional tobacco cigarettes, especially regarding the levels of sNOX2-dp (56). NOx are major air pollutants from combustion engines.
	Malondialdehyde (MDA)	Cancer	After stopping smoking, blood MDA levels reduce to healthy non-smokers' levels in 12 weeks and slightly decreases in those who only reduce smoking (58).
Inflammation markers			Local response to cellular injury that is marked by capillary dilatation, leukocytic infiltration, redness, heat, pain, swelling, or loss of function and that serves as a mechanism initiating the elimination of elimination of foreign substances and for healing damaged tissue (13)
	White blood cell count (WBC)	Cancer, CVD, Respiratory	A marker of systemic inflammation. Sensitive to tobacco exposure effects; WBC counts are positively correlated with smoking status and serum cotinine levels (52). Levels reduced when smokers switch to other tobacco products (13). Associated with the severity of COPD (59). Independent predictor of coronary heart disease and cancer death (60).

Category	Measures	Associated health risks	Other details
	C-Reactive Protein (CRP)	Cancer, CVD, Respiratory	An acute-phase, non-specific, systemic marker of inflammation (13). Correlates with vascular inflammation and lipid levels. High-sensitivity CRP (hsCRP) prevalence highest in dual users and tobacco smokers. No difference between non-smokers and EC
	Interleukin-6 (IL6)	Cancer, CVD, Respiratory	An inflammatory biomarker upstream of C-reactive protein (13). Involved in inflammation, infection responses and the regulation of metabolic, regenerative, and neural processes (52)
	Interleukin-8 (IL8)	Cancer, CVD, Respiratory	Interleukin 8 (IL-8) is a chemokine which is involved in the chemotaxis of neutrophils. IL-8 can be secreted by any cells involved in the innate immune response. The most important origin of IL-8 are macrophages (53).
	Tumour necrosis factor (TNF) alpha	Cancer, CVD, Respiratory	Results as an inflammatory response; is produced mainly in macrophages and regulates immune cells (53).
	Soluble intercellular adhesion molecule-1 (sICAM1)	CVD	A glycoprotein that is expressed in response to injury or inflammation of the endothelia. Levels reduced when smokers switch to other tobacco products (13).
	Fibrinogen	Cancer, CVD, Respiratory	Major coagulation protein in blood by mass, the precursor of fibrin, and an important determinant of blood viscosity and platelet aggregation. Low specificity for predicting CVD risk (13)

Category	Measures	Associated health risks	Other details
	Prostaglandin E2 Metabolite (PGE-M)	Cancer	PGE-M is associated with a number of cancers including colorectal, lung, breast, and head and neck cancers; increased levels have been observed in smokers in a limited number of studies (13)
	Monocyte chemoattractant protein-1 (MCP-1)	CVD	Also termed CCL2 (C-C motif ligand 2). MCP-1 is a potent chemotractant for monocyte, basophils, and memory T cells that plays an essential role in the pathogenesis of cardiovascular diseases. High levels are associated to CVD and hypertension.
Endothelial function markers			An imbalance between vasodilating and vasoconstricting substances produced by (or acting on) endothelial cells and may participate in the elevation of blood pressure and can play a role in hypertension-related vascular damage (13).
	Flow mediated dilation (FMD)	CVD	Non-invasive assessment of endothelial function in which the increase in arterial diameter, because of the reactive hyperaemia, is compared with the baseline diameter and expressed as a percentage of this baseline diameter. Measured by brachial artery ultrasound imaging. Pooled multivariate analysis shows 1% improvement in FMD translates to 13% reduction in CV events (61).

Category	Measures	Associated health risks	Other details
	von Willebrand factor	CVD	A high molecular weight pro-coagulant product of the endothelium and increased levels are found in atherosclerosis. No trend observed for increasing cigarettes per day.
	E-selectin	CVD	Vascular adhesion molecule that mediates the adhesion of neutrophils to activated vascular endothelium.
	P-selectin	CVD	Vascular adhesion molecule that mediates the adhesion of myeloid cells to activated endothelium and the adhesion of platelets to monocytes and neutrophils. Long-term smoking cessation may lead to lower levels.
	Nitric oxide	CVD	Oxidants uncouple and reduce nitric oxide, an effect considered to be the hallmark of endothelial dysfunction leading to impaired vasodilatation. Endogenous nitric oxide is highly reactive and difficult to measure directly.
	Endothelial progenitor cells (EPCs) (Circulating Angiogenic Cells)	CVD	EPCs likely derive from bone marrow, are recruited to the blood on injury, and have been found to promote the growth of blood vessels in vivo and to form capillary tubes in two-dimensional cultures.
	Microvesicles (MVs)	Cancer, CVD	A type of extracellular vesical involved in intercellular communication and transportation or mRNA and proteins between cells.

Category	Measures	Associated health risks	Other details
			A series of progressive, overlapping events, triggered by exposure of the platelets to subendothelial tissue; these events include shape change, adhesiveness, aggregation, and release reactions; when carried through to completion, these events lead to the formation of a stable hemostatic plug (13).
Other markers	Platelet activation	CVD	Excessive and persistent platelet activation contributes to inflammation and the development of atherothrombosis. On activation, platelets release several constituents stored in their dense and alpha granules, such as platelet factor 4 (PF4), D-dimer, fibrinogen, selectins, and homocysteine.

Notes: COPD—chronic obstructive pulmonary diseases; CVD—cardiovascular.

Secondary outcomes

Associations between vaping and disease incidence, progression and disease endpoints: we reviewed studies reporting on objectively measured effects of vaping on cancers, respiratory conditions (for example, asthma, COPD), cardiovascular diseases (for example, hypertension, stroke) and other diseases (for example, diabetes, oral/dental, reproductive and developmental effects).

Nicotine: we reviewed studies reporting on the pharmacokinetic effects of vaping compared with smoking and the role nicotine plays in the health risks of vaping.

Flavours: we also reviewed studies reporting on the effect exposure to flavourings in vaping products, with or without nicotine have on health.

Poisonings, fires and explosions associated with vaping products: we narratively reviewed studies reporting on incidents associated with misuse or malfunction of vaping products.

Search methods for identification of studies

We searched the following databases on 14 July 2020 and updated the search on 1 July 2021:

- CINAHL (EBSCO)
- Embase (Ovid)
- MEDLINE (Ovid)
- PsycINFO (Ovid)

The search was restricted to studies published since 1 August 2017—the last date included in the literature search conducted by the National Academies of Sciences, Engineering, and Medicine for their evidence review on public health consequences of e-cigarettes (62) and in the literature search for our 2018 evidence review of e-cigarettes and heated tobacco products (4). The search date range also minimised the potential of meta-analysing data from studies that had explored early generation and outdated vaping products.

The search terms were: ‘electronic cigarettes’, ‘vaping’, ‘e-cig*’, ‘electronic cig*’, ‘ENDS AND nicotine’, ‘electronic nicotine delivery system*’, ‘nicotine AND (vaping* OR vape* OR vapor* OR vapouris*)’. The full search strategies used for each database are provided in table 5.

Table 5. Search strategies for different electronic databases

Database	Search strategy
CINAHL (EBSCO)	(MH “Electronic Cigarettes”) OR (MH "Vaping") OR (e-cig*) OR (electronic cig*) OR (ENDS AND Nicotine) OR (electronic nicotine delivery system*) OR ((Nicotine) AND (Vaping* OR Vape* OR Vapor* OR Vapouris*))
MEDLINE (Ovid)	1. Electronic Nicotine Delivery Systems/ 2. Vaping/ 3. e-cig*.tw,kw. 4. electronic cig*.tw,kw. 5. (ENDS and nicotine).tw,kw. 6. electronic nicotine delivery system*.tw,kw. 7. vaping.tw,kw. 8. vape*.tw,kw. 9. (nicotine and (vapor* or vapouris*)).tw,kw. 10. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9
Embase (Ovid)	1. electronic cigarette/ or electronic cigarette vapor/ 2. vaping/ 3. e-cig*.tw,kw. 4. electronic cig*.tw,kw. 5. (ENDS and nicotine).tw,kw. 6. electronic nicotine delivery system*.tw,kw. 7. vaping.tw,kw. 8. vape*.tw,kw. 9. (nicotine and (vapor* or vapouris*)).tw,kw. 10. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 11. (conference abstract or conference paper or conference proceeding or "conference review").pt. 12. 10 not 11
PsycINFO (Ovid)	1. electronic cigarettes/ 2. e-cig*.tw. 3. electronic cig*.tw. 4. (ENDS and nicotine).tw. 5. electronic nicotine delivery system*.tw. 6. vaping.tw. 7. vape*.tw. 8. (nicotine and (vapor* or vapouris*)).tw. 9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8

Studies identified in different databases in the initial search were merged and de-duplicated using EndNote and imported to Rayyan systematic review management website (63) for title and abstract screening and then into Covidence systematic review management software (64) for full-text screening and data extraction. The update of the initial search was conducted following the process described by Bramer and Bain (65).

Data collection and analysis

Selection of studies

Two review authors (of ES, ET, KE and RC) independently screened titles and abstracts of studies identified in the initial and the update searches on Rayyan website. Discrepancies were resolved through discussion or with support from a third review author (LB).

Two review authors (of ES, ET, KE) independently screened full texts of the studies that had been included after the title and abstract screening. Discrepancies were resolved through discussion or with support from a third review author (LB). Full-text screening was conducted using Covidence systematic review management software (64).

Data extraction and management

We designed 2 data extraction forms—one for study characteristics in Covidence software (that is, information about study authors, funding, study design, participants, interventions, comparison groups, measures and methods for outcome assessment, authors' conclusions, and study limitations) and one for study results in Microsoft Excel spreadsheet (that is, quantitative measures of study outcomes as reported in publications and results of authors' conducted statistical testing).

Two review authors (ES, ET) independently extracted data from included studies, and any discrepancies were resolved with support from a third review author (DR, AMcN).

Assessment of risk of bias

We used different risk of bias assessment tools depending on the study design. Two authors (of AMcN, DR, ES, ET, LB) independently assessed risk of bias and resolved discrepancies through discussion. The following tools were used:

1. Randomised controlled trials: the revised Cochrane risk of bias tool RoB2 (66).

2. Cross-over studies: the RoB2 tool for cross-over trials (66).

3. Non-randomised longitudinal studies: the Cochrane risk of bias in non-randomised studies of interventions tool ROBINS-I (67).

4. Cross-sectional studies: the BIOCROSS quality assessment tool for cross-sectional studies reporting biomarker data, with minor adjustments to make the tool appropriate to

assessing vaping research (68). The overall BIOCROSS scores range from 0 to 20, with higher scores indicating lower risk of bias.

5. Poisonings, fires and explosions: the Joanna Briggs Institute critical appraisal checklists for case reports and cross-sectional studies (69) and for case series studies (70).

The risk of bias assessments of included studies are provided in the appendices.

We also extracted data on funding sources for each included study as it has been reported in publications. The study funding information is available in the appendices (table 5).

Measures of exposure to biomarkers

Summary tables

We summarised quantitative findings for the main outcomes—biomarkers of nicotine and potential toxicant exposure and biomarkers of potential harm to health and included them in separate tables.

Tables and figures of biomarkers of nicotine and potential toxicant exposure

For the biomarkers of exposure results, we separated tables by the category of biomarker of exposure (see table 3; for example, nicotine, volatile organic compounds, tobacco-specific nitrosamines, other potentially toxic compounds, carbon monoxide and metals), biosample type (for example, urine, blood, saliva, hair) and study design (for example, randomised controlled trials, cross-over studies, non-randomised acute exposure and longitudinal studies, and cross-sectional studies). We summarised findings from experimental and quasi-experimental studies (including randomised controlled trials, cross-over studies, non-randomised acute exposure and longitudinal studies) and from observational studies (cross-sectional studies) in separate tables to adjust the representation of results to the study design (for example, for studies that assessed participants at least twice, to highlight within- and between-group differences).

In tables summarising results from RCTs, cross-over and longitudinal studies, we provide general study characteristics (author, year, country where a study has been conducted), length of the longest follow-up, describe the recruited sample (sample size, smoking/vaping status at recruitment, demographic characteristics) and define intervention groups (details of assigned vaping, dual use, smoking, non-use and other study groups), and then provide results for each of the defined intervention groups at the last follow-up (group size at the last follow-up, quantitative biomarker value and measurement unit).

We calculated average within-group percentage changes from baseline to the last follow-up as:

((Follow-up level) / (Baseline level) - 1) * 100

We show up or down arrows to indicate the direction of change. If a within-group change was reported as statistically significant in a publication, we emboldened the percentage change value and arrow. Statistically significant between-group differences at the last follow-up were indicated by superscripts denoting different groups. We indicated only between-group differences at the last follow up that were reported in publications; some studies did not statistically compare between-group differences or only compared some but not all study groups.

In tables summarising results from cross-sectional studies, we provide general study characteristics (author, year, country where a study has been conducted), describe the overall recruited sample (sample size, demographic characteristics) and define study groups (subgroup sizes, definitions for vaping, dual use, smoking and non-use), and then provide results for each of the defined groups (quantitative biomarker value and measurement unit). To compare biomarker levels between vaping product (VP) users and other study subgroups, we calculated biomarker level ratios as:

(Level in VP group) / (Level in comparison group)

A ratio below 1 indicates that a biomarker level in the VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in the VP group was higher than in a comparison group. If between-group differences were statistically tested in a publication, we emboldened the cells of subgroups that were compared. We also indicated statistically significant between-group differences as they were reported in a publication by superscripts denoting different groups; if between-group differences were tested but not found, then we only emboldened cells of groups that were compared.

In figures of cross-sectional studies that included 3 comparison groups (vaping product users, smokers and non-users), we visually compare biomarker levels between vaping product users and non-users versus biomarker levels among smokers. Biomarker levels among smokers are set to 100% and relative biomarker levels within vaping product users or non-users' groups are calculated as:

((Level in VP / non-user group) / (Level in smokers)) * 100

Tables of biomarkers of potential harm to health

For the findings about biomarkers of potential harm to health, we separated tables by the associated health risk category (see table 4; for example, respiratory, cardiovascular, cancer and other biomarkers cutting across several health systems—oxidative stress, inflammation, endothelial function and other markers). In the summary tables, we provide general study characteristics (author, year, country where a study has been conducted),

study design and length of the longest follow up if a study was not cross-sectional), describe the recruited sample (sample size, smoking/vaping status at recruitment, demographic characteristics), define cross-sectional or intervention groups (details of assigned vaping, dual use, smoking, non-use and other study groups), list outcomes of interest and provide results for these outcomes as reported in the study publication. In the column summarising study findings, we include both within- and between-group changes and statistical test results as they were reported in publications.

Meta-analysis

Selecting studies for meta-analysis

Due to methodological heterogeneity of the included human studies that measured levels of potential toxicant, carcinogen and potential harm to health biomarkers, we developed an algorithm to assess whether to conduct meta-analyses (table 6).

Table 6. Steps for selecting studies for meta-analysis comparing between-group differences in vapers, smokers and non-users

Filter step	Description
1. Comparison groups	Include studies that have at least 2 out of 3 following comparison groups: vapers, smokers, non-users.
2. Clear definition of baseline sample (or a sample if study is cross-sectional)	Include studies that have clearly defined initial samples in terms of smoking/vaping frequency to be able to identify exclusive users (for example, exclude studies that define vapers as vaping and occasionally smoking). Bio-verification is not necessary if sample's smoking/vaping characteristics are defined clearly. Exclude studies that only define vapers' groups as vaping less than weekly—less frequent vaping might underestimate exposure to most toxicants that have shorter half-life characteristics. Initial sample characteristics can serve as a comparison group—for example, smokers at baseline who switch to vaping only as a cross-over condition.
3. Clear definition of follow-up groups (for RCTs, cross-over studies and non-randomised longitudinal studies)	Clearly defined follow-up groups in terms of minimum smoking/vaping frequency after randomisation (RCTs), cross-over conditions or participants' groups at follow up (non-randomised longitudinal studies). Exclude studies that only define vapers' groups as vaping less than weekly—less frequent vaping might underestimate exposure to most toxicants that have shorter half-life characteristics. The step is not relevant for cross-sectional studies.

Filter step	Description
4. Adherence to study groups	For RCT, cross-over and non-randomised longitudinal ad libitum use studies, analysis of vapers or non-users' group at follow-ups should consider the possibility of them continuing to smoke. A study analysing follow-up outcomes should state that participants in vapers or non-users' groups were not smoking, either by self-report or by bio-verification. If some participants in vapers or non-users' groups are non-adherent at follow-up (that is, were smoking), exclude studies that analyse vapers or non-users' follow-up results as uniform groups (similar to intention-to-treat analysis) and include studies that account for participant smoking and analyse follow-up groups as adherent and non-adherent participants (similar to per-protocol analysis). The step is not relevant for cross-sectional studies.
5. Data provided for baseline and follow-ups in geometric or arithmetic means and 95% CI, SE, SEM or SD.	For meta-analysis, only data that can be log-transformed are required (71). Exclude if data are reported in graphs, as a difference from baseline or as median values. If a study reported mean difference between groups in log scale, these results can be used in meta-analysis without log-transformation.
6. Data source	Where multiple studies have been published using the same data set (for example, PATH, NHANES), the study with the largest sample size will be selected for data extraction.

Notes: 95% CI—95% confidence intervals; RCT—randomised controlled trial; SD—standard deviation; SE—standard error; SEM—standard error of the mean.

For biomarkers of exposure to nicotine and potential toxicants, we pooled data for meta-analysis from studies that reported on the same biomarker collected in the same bio-sample (for example, urine, blood, saliva, hair) and which was analysed by the same methodology (for example, ELISA, LC-MS/MS). For biomarkers measured in urine, we only pooled data from studies reporting creatinine-adjusted urinary levels of a biomarker. For biomarkers of potential harm to health, we used the same study inclusion criteria (table 6) and only pooled data for meta-analysis from studies that employed similar biomarker measures. Where appropriate, we pooled biomarkers of potential harm to health data from RCTs and cross-over trials (for example, respiratory or cardiovascular outcomes), conducting separate meta-analyses for outcomes after acute, short- to-medium or long-term exposures.

Meta-analysis of pooled data

From studies that were assessed eligible for meta-analysis, we extracted continuous measures of mean and variance biomarker data and calculated mean differences on the log-transformed scale (LMD) with 95% confidence intervals (95% CI) using a method described by Higgins and others (71). We assessed log-transformed mean differences

between vapers and smokers or between vapers and non-users. We used the Review Manager software to pool the LMDs (using a generic inverse variance random-effects model) and present the pooled data estimates (72). To better communicate the log-transformed between-group mean differences in meta-analyses, we also reported geometric mean ratios (GMR) and associated 95% CI that allow us to evaluate the biomarker level differences between the comparison groups. The GMR and associated 95% CI were calculated by exponentiating the LMD and its 95% CI (71).

We assessed statistical heterogeneity between studies using the I^2 statistic. We further considered the consistency in the direction of the difference across included studies when the statistical heterogeneity I^2 was greater than 75%.

Summary of findings

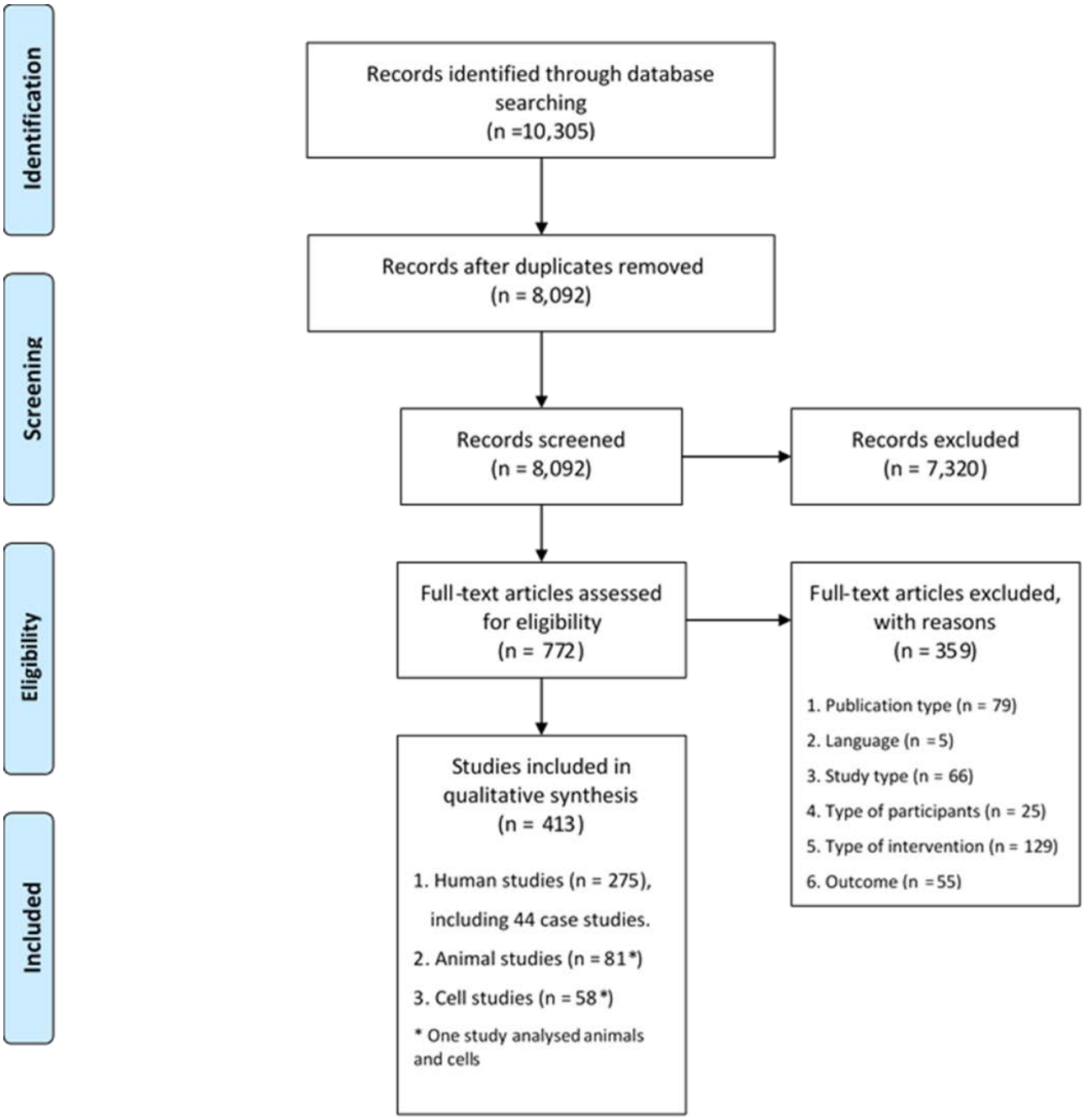
For the biomarkers of exposure to nicotine and potential toxicants chapter (chapter 7), we first summarised evidence from prior reports that explored vaping health risks due to exposure to potential toxicants (that is, reports by McNeill and others commissioned by Public Health England; the National Academies of Sciences Engineering and Medicine; and the Committee on Toxicity of Chemicals in Food Consumer Products and the Environment) and then presented our literature review findings separately for different biomarkers and summarised evidence at the end for each biomarker category (see table 1; for example, nicotine, volatile organic compounds, tobacco-specific nitrosamines, other potentially toxic compounds, carbon monoxide and metals). Where appropriate, we comment on any findings pertaining to specific populations such as adolescents or smokers with respiratory symptoms. At the end of the chapter, we provide conclusions based on the reviewed data on biomarkers of exposure and suggest further implications regarding relative and absolute health risks associated with using vaping products.

For chapters on vaping risks associated with exposure to biomarkers of potential harm (chapters 8 to 12), we first summarised evidence from the prior reports, then discussed relative study findings and, based on the reviewed data, provided conclusions and implications regarding vaping risks associated with cancer, respiratory, cardiovascular and other health.

Results: study selection

The initial and updated database searches together identified 8,092 records after duplicates were removed. Independent title and abstract screening of these records identified 772 studies for full text screening, of which 413 studies were included in the review. In total, the review summarised data from 275 studies reporting data on human participants (44 of these were case studies or case series reporting on poisonings, fires and explosions associated with vaping products), 81 studies reporting data on animal participants and 58 studies reporting on cell data, with one study covering both animal and cell data (Figure 1).

Figure 1. PRISMA flow chart of studies screening process



2.3 Heated tobacco products use and recent evidence from a Cochrane literature review

Chapter 14 of the report aimed to present recent data (described in section 2.1) on the use of heated tobacco products (HTP) in youth and adults in England and to summarise key findings from a recent Cochrane literature review (73) which assessed the effectiveness and safety of HTP for smoking cessation and the impact of HTP on smoking prevalence.

2.4 Methods: systematic literature review of vaping harm perceptions

Review questions

1. What interventions have been effective in changing vaping harm perceptions?

2. To what extent are vaping harm perceptions predictive of any changes in vaping and smoking behaviours?

Protocol registration

The protocol of the review was registered on PROSPERO.

Eligibility criteria

Types of studies

RQ1. We included quantitative experimental studies, quasi-experimental (natural experiment/pre-post) studies, trials, surveys (any mode), and observational studies that examined interventions involving communication/messaging of the harms of vaping (including EVALI) and changes in vaping harm perceptions. We only included studies that examined changes in vaping harm perceptions, either within or between individuals, and that use longitudinal or repeated methods with more than 1 time-point.

RQ2. We included longitudinal quasi-experimental studies, surveys, observational studies, and the control arms of trials/experiments that examined longitudinal associations between vaping harm perceptions and any subsequent changes in vaping and smoking behaviours. We only included studies that assessed changes in vaping and smoking behaviours within individuals. We excluded cross-sectional studies (also excluding repeated cross-sectional studies).

Both RQ1 and RQ2 (all studies). We excluded qualitative studies, case studies/series, systematic reviews/meta-analyses. We included peer-reviewed published papers and

those in press. We excluded non-peer-reviewed literature (for example posters, conference abstracts, PhD theses, pre-prints). We only included publications in English, French, and German languages.

Condition or domain being studied

We defined vaping as follows: ‘vaping’ is the act of using an e-cigarette or a vaping product. Vaping involves using a battery-powered heating element designed to vaporise a solution made of propylene glycol and/or glycerine, water and usually flavouring compounds, flavour enhancers, and nicotine (freebase or nicotine salts). This vapour (aerosol) is then inhaled. Vaping products do not contain tobacco and do not involve combustion.

We defined vaping risks (including communication, messaging, and perceptions of vaping risks) as:

- risks, harm, risk of disease, addictiveness
- risk of firsthand and secondhand use/exposure to vaping and e-cigarette emissions
- relative risk of vaping compared to smoking or other nicotine-containing products (for example, perceiving vaping as less risky or harmful to health than smoking)
- absolute risk (for example, perceiving vaping as risky or harmful to health)
- risk of nicotine
- risk of subsequent smoking initiation/uptake
- uncertainty of vaping risks

For communication/messaging of vaping risks, we also included messages about the prevention of vaping. We included messages that were targeted toward youth, adult smokers, or any other groups.

We excluded the following for communication, messaging, and perceptions of vaping risks:

- risk of smoking/cigarettes alone (that is, not in relation to vaping)
- risk of using other nicotine/tobacco products (for example, NTR, waterpipe, smokeless tobacco) alone (that is, not in relation to vaping)
- risk of cannabis vaping or vaping other illicit drugs

- harm perceptions of vaping as a reason for vaping (that is, not harm perceptions per se, but vaping because – for example – of a perception that vaping is less harmful than smoking)

Types of participants

Any people of any age.

Types of interventions (RQ1) or exposure (RQ2)

RQ1. We included studies with an intervention that involved any communication or messaging of the risks of vaping (defined above under 'Condition or domain being studied'). Interventions could include public health/education campaigns, mass media campaigns, industry funded/ affiliated campaigns, advertisements, packaging (including written or pictorial warning labels, imagery, alternative/experimental warnings or designs), any other exposure to messages.

RQ2. We included studies that assessed vaping harm perceptions (defined above under 'Condition or domain being studied').

Types of control/comparisons

Where there are comparators, the comparator/control conditions are those reported in the studies (aside from the intervention/exposure above). We also included studies with no comparators.

Types of outcomes

RQ1. We included articles that assessed changes in vaping harm perceptions (defined above under 'Condition or domain being studied') as an outcome. Changes could be measured within-person (for example, trials, experiments, longitudinal surveys) or at the population level (for example, repeated cross-sectional surveys). We only included articles that measured vaping harm perceptions before/concurrently and after exposure to the intervention, and reported changes.

RQ2. We included articles that assessed longitudinal changes in vaping and smoking behaviours. Vaping is defined above under 'Condition or domain being studied'. Smoking is defined as combustible tobacco cigarette smoking. We only included articles that measured changes in vaping and smoking behaviours within-person. We only included articles that measured behaviours before/concurrently and after harm perceptions were measured, and reported changes. We included articles that reported any changes in vaping and smoking behaviours, including but not limited to vaping or smoking initiation or uptake, vaping or smoking cessation or reduction, switching from vaping to smoking, switching from smoking to vaping, increases or decreases in consumption or frequency of vaping or smoking.

Where articles measured the associations between exposures and outcomes but did not report them in the results, we contacted the study's authors to request this information. We included articles where the authors provided this information.

Search strategy, information sources and study selection

The search strategy was adapted from those used in our previous reports (1-5) and the health effects review to include harm perception terms (Appendix 1). Embase, PsycINFO, Medline, CINAHL, and Scopus databases were searched on 15 April 2021 for articles published since January 2007 (when e-cigarettes were introduced to the UK market) to present. The search was later updated to include articles published between 15 April 2021 and 1 July 2021.

Search terms were adapted for each database to align with differences in keyword terms and syntax requirements. The full search terms used for each database are included in Appendix 1.

The outputs of the search were merged and de-duplicated using Endnote and imported to Covidence (64), a systematic review management software.

Two reviewers independently screened all titles, abstracts, and full texts. Conflicts were resolved by discussion and consulting a third reviewer. Conflicts over 'reasons for exclusion' were resolved according to the hierarchy of exclusion criteria, with the reason highest on the hierarchy being selected.

Where articles measured the associations between exposures and outcomes but did not report them in the results, authors were contacted to request this information.

Data collection process and data items

All data were extracted by one reviewer and data from 10 articles were checked by a second reviewer. The quality of extracted data was considered good. Discrepancies among these 10 articles were resolved between the 2 reviewers, and the team consulted if unresolved.

The summary of characteristics for each study included author, year of publication, country, setting, data collection period, participants, funding and conflicts of interest (including tobacco industry funding/affiliation), and risk of bias. The summary of methods and findings for each study included intervention/exposure, follow-up, analyses, outcome measurement, and associations between intervention/exposure and outcome.

Quality and risk of bias assessment

Risk of bias of the included studies was assessed using different tools dependent on the study design. For randomised studies we used the Cochrane risk of bias tool for randomized trials (Version 2) (66). For non-randomized studies of interventions, we used the Risk of Bias in Non-randomized Studies of Interventions tool (ROBINS-I) (67). For cohort studies, we used an adapted 5-star Newcastle Ottawa Scale (NOS) (74, 75); scores range from 0 to 5 stars, with 3 or fewer stars indicating high risk of bias. For repeat cross-sectional studies, we used an adapted 8-star version of the NOS for cross-sectional studies (76) with higher scores indicating higher risk of bias.

Data synthesis

All findings are narratively synthesised and described. Narrative synthesis was used because of the heterogeneity of the methods, interventions/exposures, outcomes, and analyses, as per the PROSPERO registration.

The PRISMA flowchart for this review is shown in chapter 15.

2.5 Overall conclusions

For chapters based on our systematic literature reviews of the health risks of vaping and vaping harm perceptions and on the 2022 Cochrane review on HTP (73), which we summarise in chapter 14, we provide overall conclusions broadly following the definitions of level of evidence provided by NASEM (table 7).

Table 7. Definitions of levels of evidence as reported in the NASEM report (62)

Evidence level	Definition
No available evidence	There are no available studies; health endpoint has not been studied at all. No conclusion can be made.
Insufficient evidence	There are mixed findings or a single poor study. No conclusion can be made because of substantial uncertainty due to chance, bias, and confounding factors
Limited evidence	There are supportive findings from fair-quality studies or mixed findings with most favouring one conclusion. A conclusion can be made, but there is significant uncertainty due to chance, bias, and confounding factors.
Moderate evidence	There are several supportive findings from fair-quality studies with few or no credible opposing findings. A general conclusion can be made, but limitations, including chance, bias, and confounding factors, cannot be ruled out with reasonable confidence.
Substantial evidence	There are several supportive findings from good-quality observational studies or controlled trials with few or no

Evidence level	Definition
	credible opposing findings. A firm conclusion can be made, but minor limitations, including chance, bias, and confounding factors, cannot be ruled out with reasonable confidence.
Conclusive evidence	There are many supportive findings from good-quality controlled studies (including randomized and non-randomized controlled trials) with no credible opposing findings. A firm conclusion can be made, and the limitations to the evidence, including chance, bias, and confounding factors, can be ruled out with reasonable confidence.

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3 Vaping among young people

3.1 Objective

This chapter summarises survey data on vaping among young people in England. The focus is on vaping, with smoking data also presented where comparisons are appropriate and illustrative. As well as reporting on vaping prevalence overall, this chapter summarises vaping by socio-demographic characteristics. It also covers reasons for use, product preferences and sources of vaping products. The chapter also briefly presents prevalence of use of nicotine pouches and smokeless tobacco among young people in England.

3.2 Surveys

Continuing a decline of available data noted in our 2021 report (1), there are fewer available data on young people compared with previous years for the following reasons:

1. The largest survey used in our 2020 report (2) was the Smoking, Drinking and Drugs (SDD) survey—this survey was suspended due to coronavirus (COVID-19) and therefore there are no new SDD data available for the present report.

2. The Health Survey for England runs every year; however, fieldwork was suspended due to COVID-19 and so data are not available for this report.

Action on Smoking and Health – Youth (ASH-Y) surveys (11 to 18 year olds) have been used in our previous reports. These surveys are conducted online and designed to be nationally representative. We presented the ASH-Y 2020 data in our last review, so in this chapter we present data from the 2021 survey. However, in May 2022 as we were finalising our report, we became aware that ASH was to publish a report from their 2022 ASH-Y Survey findings around the same time as we would be publishing our report. For consistency across 2 reports, we therefore incorporated top-line smoking and vaping prevalence data from the 2022 ASH-Y survey (age 11 to 18, sample size for England = 2,259, data collected February to March 2022) into this chapter. As the 2022 ASH-Y survey data also identified a change in the types of vaping products used, and in line with issues identified by trading standards officers about disposable vaping products discussed in our Introduction chapter, we also included the 2022 ASH-Y data on types of vaping products used. Given time constraints, we were unable to include other 2022 ASH-Y data but the full report from the 2022 ASH-Y survey will be available on the ASH website.

As in our 2021 report (1), we also complement and contrast ASH-Y 2021 by including data from the International Tobacco Control Policy Evaluation Project (ITC) Youth Tobacco and Vaping survey 2021, which is also conducted online. The ITC Youth survey is a large

survey of 16 to 19 year olds that is weighted to ensure that the sample matches national benchmarks for age, sex and region. It has been running annually since 2017 with supplementary biannual waves in 2020 and 2021. For continuity and alignment with data on young people in our previous reports, we predominantly report ASH-Y data in this chapter, but supplement this with ITC Youth survey data where appropriate. The ITC Youth survey includes more in-depth information on vaping products used including e-liquids, as well as including a slightly older demographic than ASH-Y.

3.3 Smoking and vaping prevalence among young people in England

Table 1 presents the latest available data on smoking and vaping among young people in England from the ASH-Y and ITC Youth surveys. Current smoking prevalence (people who smoked sometimes but less than weekly, as well as those who smoked more than once a week) was 4.1% in 2021 and 6.0% in 2022 for 11 to 18 year olds (ASH-Y), with 83.5% having never tried smoking in 2021 and 80.2% having never tried smoking in 2022. In 2021, the 4.1% of current smokers were made up of those who smoked less than once a week (2.1%), those who smoked between 1 to 6 cigarettes a week (0.9%) and those who smoked more than 6 cigarettes a week (1.1%). In 2022, the 6.0% of current smokers were made up of those who smoked less than once a week (3.2%), those who smoked between 1 to 6 cigarettes a week (1.2%) and those who smoked more than 6 cigarettes a week (1.6%).

Smoking prevalence was lower in 2021 than previous ASH-Y figures of 6.3% for 2019 and 6.7% for 2020. This was possibly due to the effects of the COVID-19 pandemic such as reduced peer contact, greater time spent under parental supervision or limited access to cigarettes. Smoking prevalence in 2022 was similar to previous years, so it is currently unclear whether the 2021 data differed due to COVID-19.

In February 2021 the ITC Youth survey reported 7.9% of 16 to 19 year olds currently smoked cigarettes (that is, they had smoked more than 100 cigarettes in their life and had smoked in the past 30 days), with 58.3% saying they had never smoked. Data from 2019 and 2020 suggest a slight decline since 2020 (table 1).

In the ASH- Y survey, current vaping prevalence among young people who vaped at least monthly was 4.0% in 2021 and 8.6% in 2022, compared with 4.8% in both 2019 and 2020. In 2021, the 4.0% of current vapers were made up of young people who vaped sometimes but not more than once a month (1.7%), more than once a month but less than once a week (0.9%), more than once a week but not daily (0.7%) and those who vaped daily (0.7%). In 2022, the 8.6% of current vapers were made up of young people who vaped sometimes but not more than once a month (2.6%), more than once a month but less than once a week (1.9%), more than once a week but not daily (1.8%) and those who vaped

daily (2.3%). The proportion of young people who had never vaped in 2021 was 86.3%. The proportion of young people who had never vaped in 2022 was 80.9%.

In the February 2021 ITC Youth survey 9.1% of 16 to 19 year olds currently vaped (that is, they had vaped more than 10 days in their life and vaped in the past 30 days), and 12.2% had vaped in the past 30 days compared with 12.6% and 14.0% respectively in 2019 and 2020. The proportion who had never vaped was 57.4% in 2021, suggesting a slight decline from 63.9% in 2019 and 58.3% in 2020 (table 1).

The differences between the 2 surveys in vaping and smoking estimates for 2021 are likely attributable to the different age ranges covered by each survey, as well as differing definitions of smoking and vaping (see table 1 notes). ASH-Y includes young people aged 11 to 18 years old, whereas the ITC survey includes young people aged 16 to 19 years old. Vaping was more prevalent among older adolescents: in the ITC survey, 8.2% of 16 and 17 year olds, 10.2% of 18 year olds and 14.7% of 19 year olds reported current vaping (Figure 1). These are comparable with 6.4% of 16 and 17 year olds and 9.3% of 18 year olds in the ASH-Y data (Figure 1). Similarly, in the ITC survey, 6.1% of 16 and 17 year olds and 8.1% of 18 year olds are current smokers, broadly comparable to current smoking estimates of 6.6% for 16 and 17 year olds and 10.7% for 18 year olds from the ASH-Y survey (table 2). Ultimately, the inclusion of the 11 to 15 year olds in the ASH-Y survey decreases estimates of smoking and vaping, whereas the inclusion of 19 year olds in the ITC survey increases smoking and vaping estimates.

Table 1. Current smoking and vaping prevalence among young people in 2 national surveys, England (ASH-Y 2015 to 2022 and ITC 2019 to 2021; weighted data)

Survey	ASH-Y 2015	ASH-Y 2016	ASH-Y 2017	ASH-Y 2018	ASH-Y 2019	ASH-Y 2020	ASH-Y 2021	ASH-Y 2022	ITC August/Sept 2019	ITC Feb/ March 2020	ITC Feb/ March 2021
Unweighted sample size	1,926	1,999	2,260	2,011	2,173	2,168	2,151	2,259	3,493	4,265	4,224
Age	11 to 18	11 to 18	11 to 18	11 to 18	11 to 18	11 to 18	11 to 18	11 to 18	16 to 19	16 to 19	16 to 19
Smoking status %											
Never tried	77.1	80.3	76.9	78.6	79.7	80.9	83.5	80.2	62.0	54.5	58.3
Tried only[1]	11.7	9.7	10.7	10.2	9.0	8.3	8.6	8.1	31.0	35.9	32.2
Former	3.0	3.3	3.6	3.5	3.4	3.0	3.0	3.7	0.8	1.0	1.7
Current	7.1	5.2	7.8	6.1	6.3	6.7	4.1	6.0	6.2	8.5	7.9
Vaping status %											
Never tried	93.9	87.8	83.2	82.8	83.6	82.8	86.3	80.9	63.9	58.3	57.4
Tried only	4.7	9.3	10.9	12.3	9.4	10.0	8.6	9.1	23.8	25.2	24.9
Former	-	-	1.7	0.8	0.9	1.8	1.2	1.4	4.6	7.1	8.6
Current	1.2	2.5	3.5	3.5	4.8	4.8	4.0	8.6	7.7	9.4	9.1
Past 30-day	-	-	-	-	-	-	-	-	12.6	14.0	12.2

Notes: ASH-Y: Never smokers were young people who had never tried cigarettes. Tried only smokers were young people who had only ever tried smoking cigarettes once. Former smokers were young people who used to smoke sometimes but who never smoked now. Current smokers were young people who smoked sometimes but less than weekly, as well as those who smoked more than once a week. Never vapers were young people who had never tried vaping as well as those who had never heard of vaping products (e-cigarettes). Tried only vapers were young people who had only tried vaping once or twice. Former vapers were young people who used vaping products in the past but who no longer do. Current vapers were young people who vaped at least monthly.

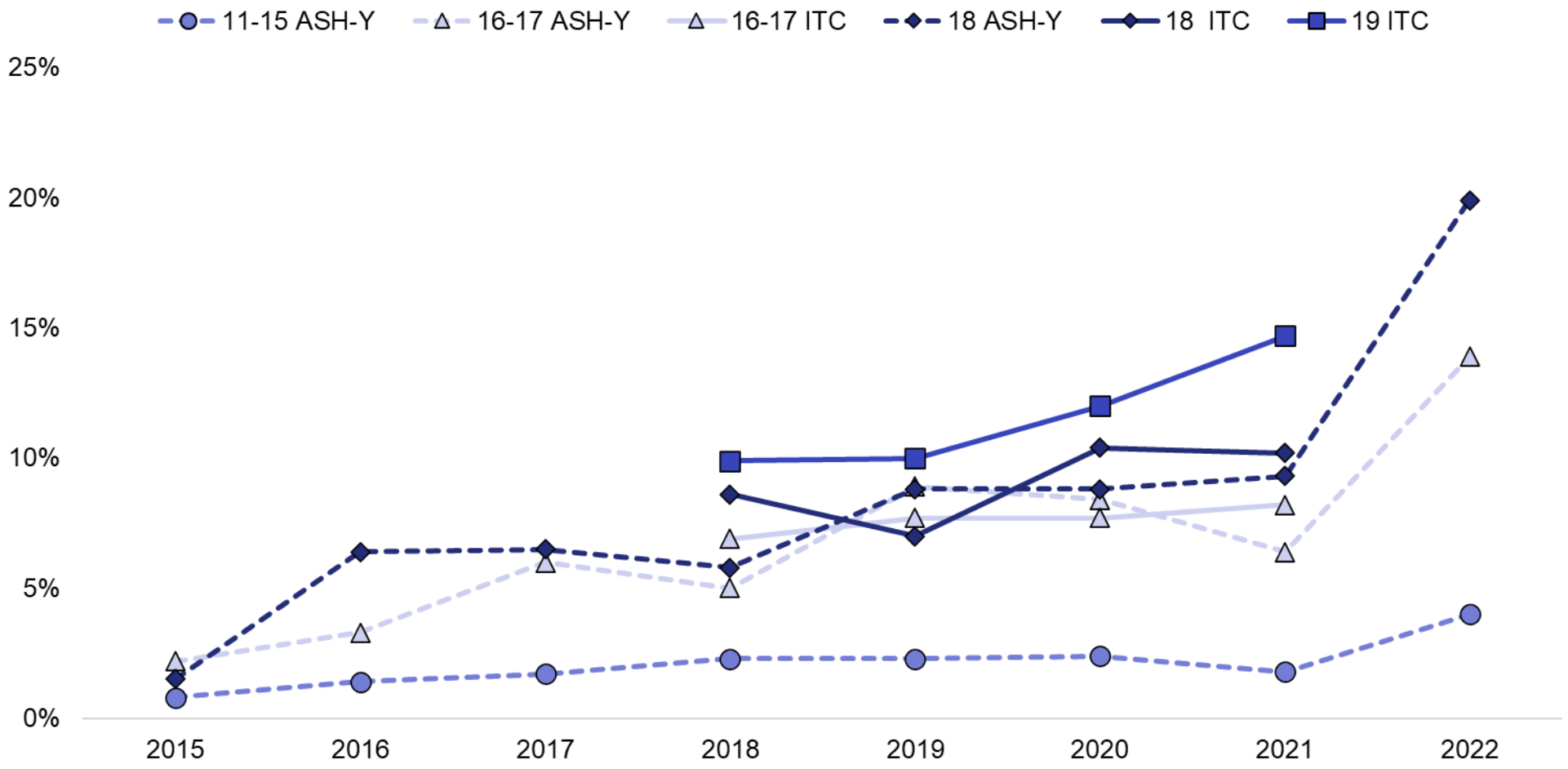
0.8% of participants in 2021 (n=17) and 2.0% (n=46) of participants in 2022 did not want to say what their smoking status was, therefore column percentages might not total 100 for smoking status.

ITC: Never smokers were young people who had never tried cigarettes. Tried only smokers (referred to as ‘Experimental smokers’ in the ITC survey) were young people who had tried cigarettes, but who had not smoked more than 100 cigarettes in their life. Former smokers were young people who had smoked more than 100 cigarettes in their life, but who had not smoked in the past 30 days. Current smokers were young people who had smoked more than 100 cigarettes in their life and who had smoked in the past 30 days. Never vapers were young people who had never tried vaping. Tried only vapers were young people who had tried vaping, but who had vaped on no more than 10 days in their life. Former vapers were young people who had vaped on more than 10 days in their life, but who had not vaped in the past 30 days. Current vapers were young people who had vaped on more than 10 days in their life and who had vaped in the past 30 days. Past 30-day vapers were young people who had vaped at least once in the past 30 days.

[1] ITC denotes ‘tried only’ as ‘experimental’ smokers.

‘-’ signifies comparable data are not available.

Figure 1. Current vaping over time among young people by age, England (ASH-Y 2015 to 2022; ITC 2018 to 2021, weighted data)



Notes: Unweighted bases ASH-Y: 2015=1,926, 2016=1,999, 2017= 2,260, 2018=2,011, 2019=2,173, 2020=2,168, 2021=2,151, 2022=2,259.

ITC: 2019=3,493, 2020=4,265, 2021=4,224.

ASH-Y: Current vapers were young people who vaped at least monthly.

ITC: Current vapers were young people who had vaped on more than 10 days in their life and who had vaped in the past 30 days.

Table 2 contains data for smoking prevalence by socio-demographic characteristics using the ASH-Y 2021 and 2022 data.

As previously discussed, the proportion of young people who were current smokers appears to increase with age, for example in 2021, 1.6% of 11 to 15 year olds smoked compared with 10.7% of 18 year olds, and in 2022 2.4% of 11 to 15 year olds smoked compared with 13.8% of 18 year olds. The 2021 and 2022 estimates for 18 year olds were lower than the equivalent estimates in 2020 (15.6%) and 2019 (16.5%), and there were few variations according to gender and region.

In 2021, the estimate for smoking prevalence by social grade was 4.6% for people from social grades A, B and C1 (ABC1) and 2.8% for people from social grades C2, D and E (C2DE). In 2022, there was little variation between ABC1 (5.8%) and C2DE (5.4%). Table 3 shows the definition of these social grades which are derived by YouGov based on the main income earner in the household.

Table 2. Smoking prevalence among young people by age, gender, region and social grade, England 2021 to 2022 (ASH-Y, weighted data)

	Never smoker, % (n)		Tried only, % (n)		Former smoker, % (n)		Current smoker, % (n)	
	2021	2022	2021	2022	2021	2022	2021	2022
Total	83.5 (1832)	80.2 (1831)	8.6 (190)	8.1 (184)	3.0 (66)	3.7(84)	4.1 (89)	6.0 (136)
Age								
11 to 15	91.0 (1238)	89.8 (1273)	4.6 (63)	5.5 (78)	2.1 (29)	1.7 (24)	1.6 (22)	2.4 (34)
16 to 17	74.1 (404)	68.7 (395)	14.1 (77)	10.8 (62)	3.9 (21)	5.2 (30)	6.6 (36)	10.8 (62)
18	65.7 (190)	56.5 (163)	17.3 (50)	15.2 (44)	5.5 (16)	10.4 (30)	10.7 (31)	13.8 (40)
Gender								
Female	83.3 (901)	79.7 (921)	8.6 (93)	8.6 (99)	3.0 (32)	3.5 (40)	4.3 (46)	6.1 (70)
Male	83.6 (931)	80.8 (909)	8.7 (97)	7.6 (85)	3.1 (35)	3.9 (44)	3.9 (44)	6.0 (67)
Region								
North	86.3 (521)	79.9 (502)	7.3 (44)	9.2 (58)	2.6 (16)	3.7 (23)	3.3 (20)	6.1 (38)
Midlands	85.3 (365)	82.4 (366)	8.2 (35)	6.3 (28)	3.0 (13)	3.2 (14)	2.8 (12)	4.3 (19)
South	81.3 (946)	79.7 (963)	9.5 (111)	8.1 (98)	3.2 (37)	3.9 (47)	5.0 (58)	6.5 (79)
Social grade								
ABC1	82.8 (1290)	81.2 (1252)	8.9 (139)	8.5 (131)	3.1 (49)	3.0 (47)	4.6 (72)	5.8 (90)
C2DE	85.1 (542)	79.6 (518)	8.0 (51)	6.5 (42)	2.7 (17)	5.4 (35)	2.8 (18)	5.4 (35)

Notes: Unweighted base 2021=2,151; 2022=2,259. Never smokers were young people who had never tried cigarettes. Tried only smokers were young people who had only ever tried smoking cigarettes once. Former smokers were young people who used to smoke sometimes but who never smoked now. Current smokers were young people who smoked sometimes but less than weekly, as well as those who smoked more than once a week. 1% of participants in 2021 (n=25) and 2.1% (n=47) of participants in 2022 did not want to say what their smoking status was, therefore row percentages might not total 100.

Table 3. Social grade classifications derived from the National Readership Survey

Social grade	Description
A	High managerial, administrative or professional
B	Intermediate managerial, administrative or professional
C1	Supervisory, clerical and junior managerial, administrative or professional
C2	Skilled manual workers
D	Semi and unskilled manual workers
E	State pensioners, casual or lowest grade workers, unemployed with state benefits only

Table 4 shows the estimates of vaping prevalence by socio-demographic characteristics and similarly indicates increased vaping prevalence with age. In 2021 1.8% of 11 to 15 year olds were currently vaping compared with 9.3% of 18 year olds, and in 2022 4.0% of 11 to 15 year olds were currently vaping compared with 19.9% of 18 year olds.

Vaping prevalence among males was 3.6% compared with 4.3% among females in 2021 and 7.9% among males and 9.2% among females in 2022. Whereas the 2021 data indicate that, as with smoking prevalence, vaping prevalence may have been higher among social grades ABC1 (4.4%) than among C2DE (3.0%), there was little variation in vaping prevalence between social grades ABC1 (8.4%) and C2DE (8.1%) in 2022 (similar to the lack of variation in smoking prevalence between social grades in 2022).

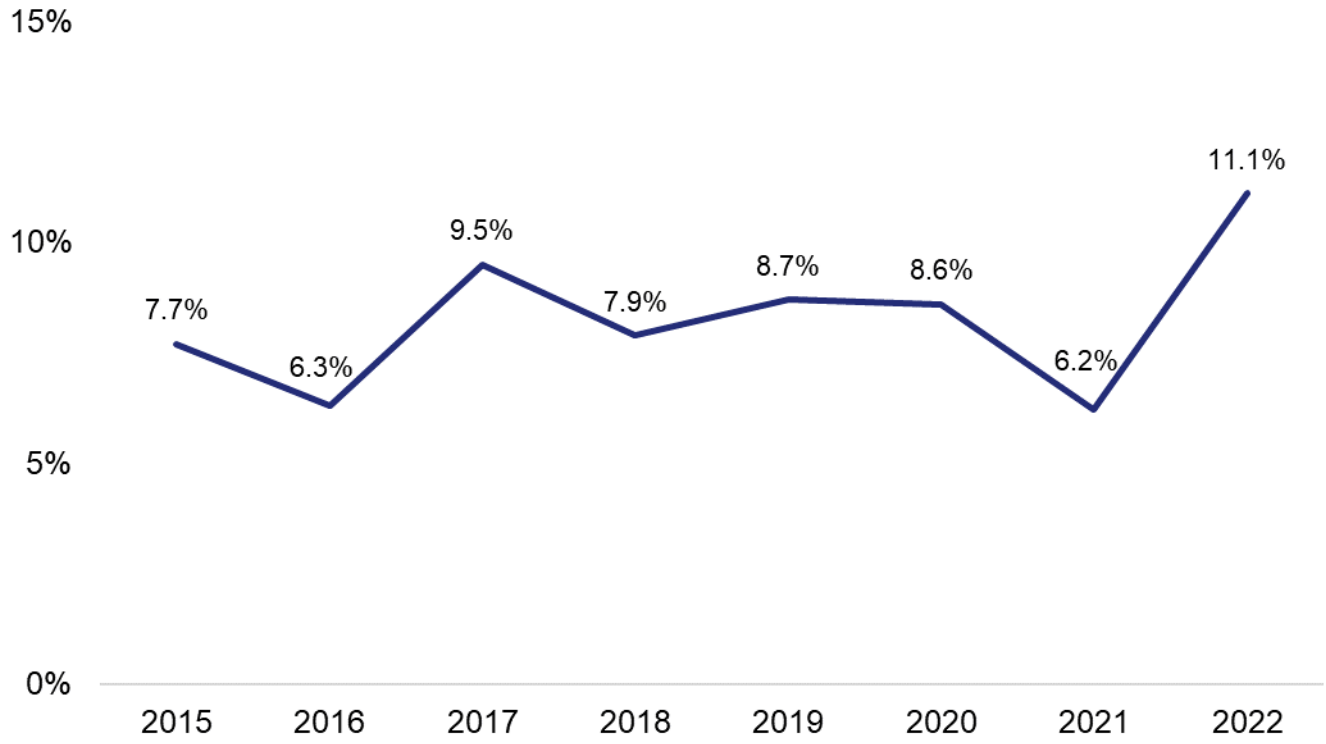
Table 4. Vaping status among young people by age, gender, region, social grade and smoking status, England 2021 to 2022 (ASH-Y, weighted data)

	Never vaper % (n)		Tried only % (n)		Former vaper % (n)		Current vaper % (n)	
	2021	2022	2021	2022	2021	2022	2021	2022
Total	86.3 (1892)	80.9 (1846)	8.6 (189)	9.1 (208)	1.2 (25)	1.4 (32)	4.0 (87)	8.6 (195)
Age								
11 to 15	93.8 (1274)	89.1 (1263)	3.8 (52)	6.3 (89)	0.5 (7)	0.6 (8)	1.8 (25)	4.0 (57)
16 to 17	76.0 (415)	71.6 (411)	15.8 (86)	11.8 (68)	1.8 (10)	2.6 (15)	6.4 (35)	13.9 (80)
18	70.2 (203)	59.1 (172)	17.6 (51)	13.9 (80)	2.8 (8)	3.4 (10)	9.3 (27)	19.9 (58)
Gender								
Female	86.7 (936)	79.7 (897)	8.2 (89)	9.4 (106)	0.8 (9)	1.6 (18)	4.3 (46)	9.2 (104)
Male	86.0 (957)	82.1 (949)	9.0 (100)	8.8 (91)	1.4 (16)	1.2 (14)	3.6 (40)	7.9 (91)
Region								
North	87.3 (528)	79.9 (501)	7.4 (45)	10.5 (66)	1.3 (8)	1.0 (6)	4.0 (24)	8.9 (56)
Midlands	87.6 (374)	82.7 (368)	9.1 (39)	7.6 (34)	0.9 (4)	2.0 (9)	2.3 (10)	7.6 (34)
South	85.2 (991)	80.8 (977)	9.1 (106)	9.0 (109)	1.1 (13)	1.5 (18)	4.6 (53)	8.7 (105)
Social grade								
ABC1	85.2 (1328)	81.3 (1253)	9.1 (142)	9.1 (140)	1.3 (21)	1.3 (20)	4.4 (68)	8.4 (129)
C2DE	89.0 (565)	81.7 (532)	7.4 (47)	8.4 (55)	0.6 (4)	1.7 (11)	3.0 (19)	8.1 (53)
Smoking Status**								
Never smoker	95.0 (1741)	91.6 (1677)	3.9 (72)	6.3 (115)	0.3 (5)	0.4 (7)	0.8 (14)	1.7 (32)
Tried only	48.9 (93)	40.2 (74)	37.9 (72)	32.6 (60)	2.1 (4)	2.7 (5)	11.1 (21)	24.5 (45)
Former smoker	36.4 (24)	32.5 (27)	31.8 (21)	13.3 (11)	13.6 (9)	10.8 (9)	18.2 (12)	43.4 (36)
Current smoker	22.5 (20)	23.5 (32)	25.8 (23)	13.2 (18)	6.7 (6)	6.6 (9)	44.9 (40)	56.6 (77)

Notes: Unweighted base 2021=2,193, 2022=2,259 (n=2,146 for social grade). Never vapers were young people who had never tried vaping as well as those who had never heard of vaping products (e-cigarettes). Tried only vapers were young people who had only tried vaping once or twice. Former vapers were young people who used vaping products in the past but who no longer do. Current vapers were young people who vaped at least monthly. Never smokers were young people who had never tried cigarettes. Tried only smokers were young people who had only ever tried smoking cigarettes once. Former smokers were young people who used to smoke sometimes but who never smoked now. Current smokers were young people who smoked sometimes but less than weekly, as well as those who smoked more than once a week. Weighted data.

** 0.8% of participants in 2021 (n=17) and 2.0% (n=46) of participants in 2022 did not want to say what their smoking status was, therefore row percentages might not total 100.

Figure 2. Combined estimates of current smoking and/or vaping over time among young people (11 to 18 years old), England 2015 to 2022 (ASH-Y, weighted data)



Notes: Participants who currently vape and/or smoke.

Unweighted bases 2015=1,926, 2016=1,999, 2017=2,260, 2018=2,011, 2019=2,173, 2020=2,168, 2021=2,151, 2022=2,259.

Current vapers were young people who vaped at least monthly.

Current smokers were young people who smoked sometimes but less than weekly, as well as those who smoked more than once a week.

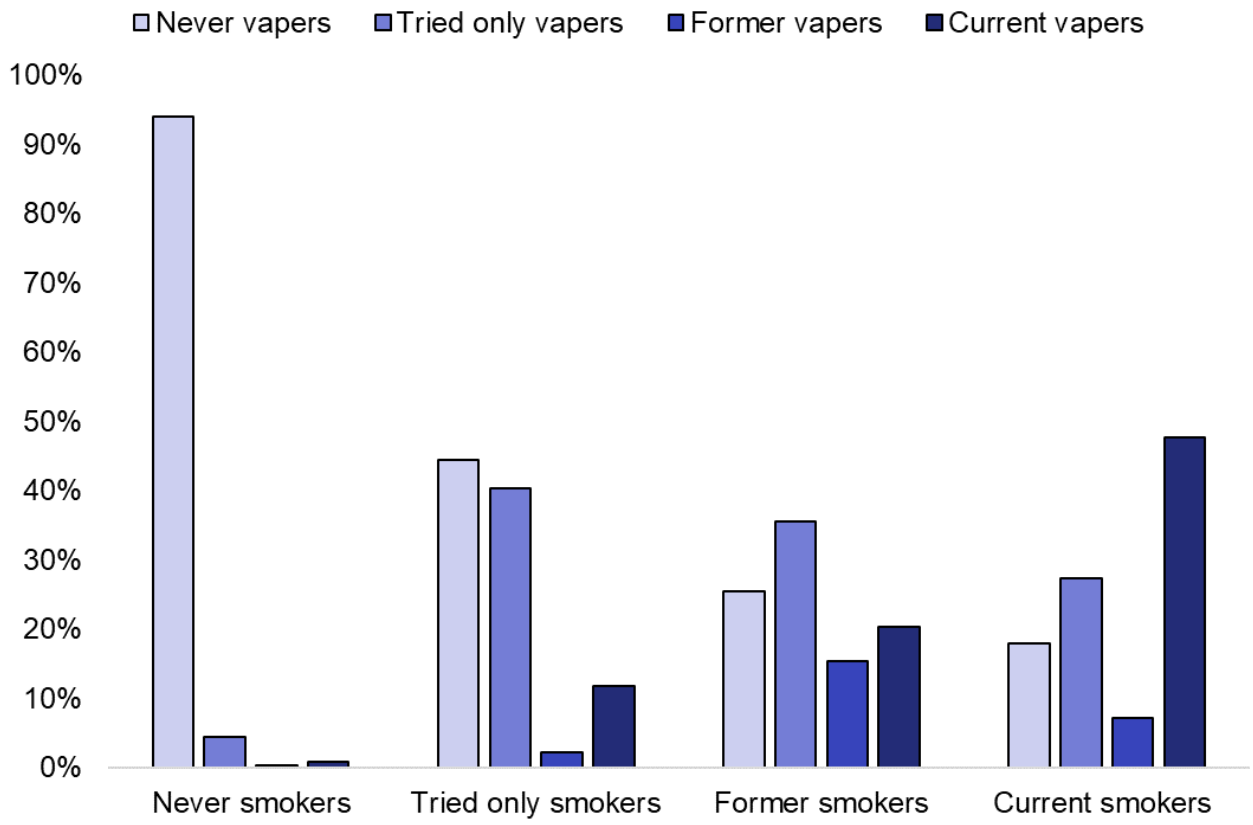
Figure 2 shows the combined estimates of current vaping and/or smoking among youth aged 11 to 18 years between 2015 and 2022. Although there has been an increase in vaping reported between 2015 and 2020 (table 1), there was little change in overall levels of vaping and/or smoking until 2020, with a decline observed between 2020 and 2021 and an increase between 2021 and 2022.

Most young people who had never smoked had also never vaped (table 4, Figure 3). ASH-Y data indicate that in 2021 95.0% and in 2022 91.6% of 11 to 18 year olds who had never smoked had also never vaped, and 0.8% of never smokers in 2021 and 1.7% of never smokers in 2022 were current vapers. In 2021, an estimated 44.9% of current smokers, and 18.2% of former smokers currently vaped. In 2022 an estimated 56.6% of current smokers, and 43.4% of former smokers currently vaped.

The proportion of smokers who were concurrently vaping was 39.2% in the 2021 ITC Youth survey, 44.9% in the 2021 ASH-Y and 56.7% in 2022 ASH-Y surveys. The ITC Youth data indicate that 81.0% of 16 to 19 year olds who had never smoked had also never vaped, and a very low proportion of never smokers reported currently vaping (0.8%). In both surveys, a high proportion of people who had tried or experimented with vaping had also tried smoking.

Figure 3. Vaping status by smoking status among young people, England 2021 (ASH-Y and ITC Youth, weighted data)

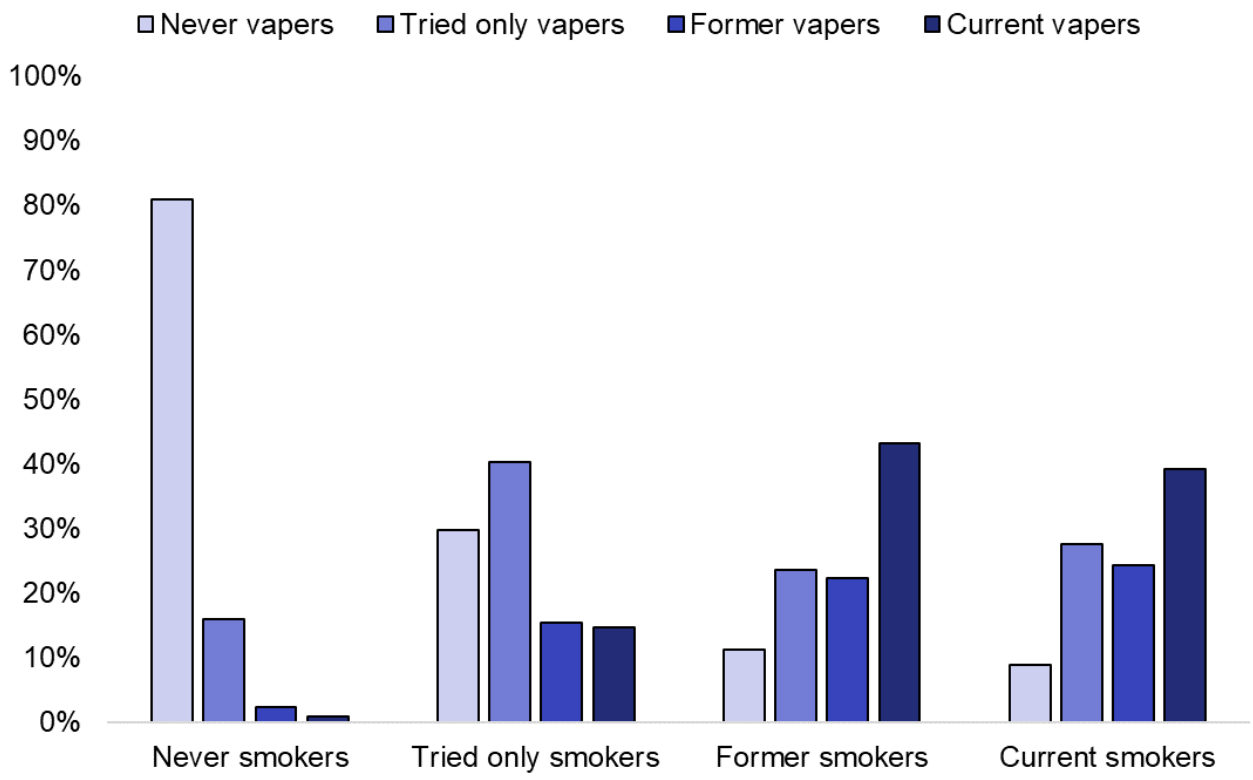
ASH-Y aged 11 to 18



Notes: Unweighted base=1,944 young people who were aware of vapes (e-cigarettes). Never smokers were young people who had never tried cigarettes. Tried only smokers were young people who had only ever tried smoking cigarettes once. Former smokers were young people who used to smoke sometimes but who never smoked now. Current smokers were young people who smoked sometimes but less than weekly, as well as those who smoked more than once a week. Never vapers were young people who had never tried vaping. Tried vaping were young people who had only tried vaping once or twice. Former vapers were young people who used vaping products in the past but who no longer do. Current vapers were young people who vaped at least monthly.

Young people who had not heard of vapes (e-cigarettes) (n=179, 8.3%) or did not know if they had heard of vapes (n=28, 1.3%) were not included.

ITC aged 16 to 19

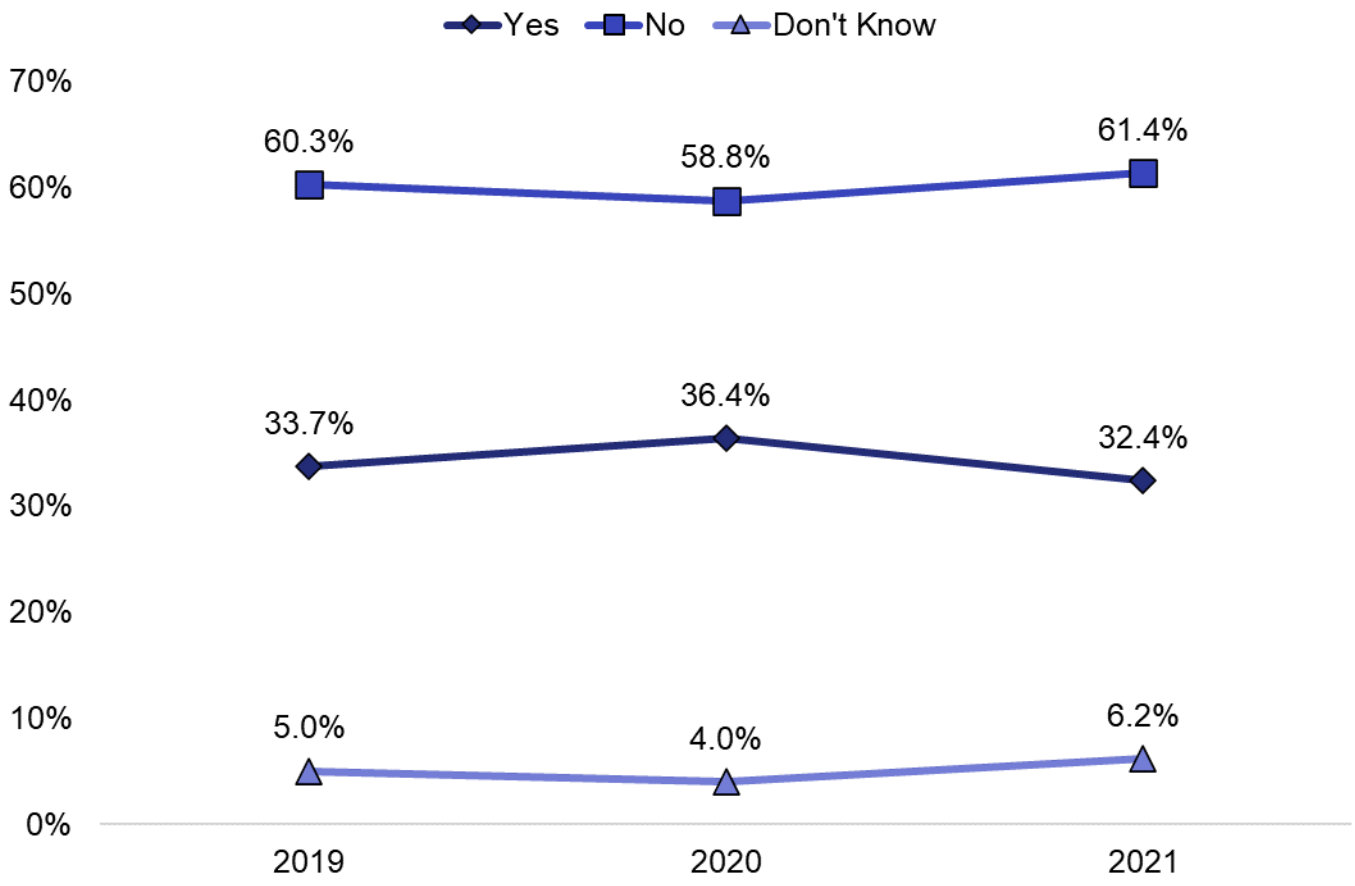


Notes: Unweighted base=4,224. Never smokers were young people who had never tried cigarettes. Tried only smokers (referred to as 'Experimental smokers' in the ITC survey) were young people who had tried cigarettes, but who had not smoked more than 100 cigarettes in their life. Former smokers were young people who had smoked more than 100 cigarettes in their life, but who had not smoked in the past 30 days. Current smokers were young people who had smoked more than 100 cigarettes in their life and who had smoked in the past 30 days. Never vapers were young people who had never tried vaping. Tried only vapers were young people who had tried vaping, but who had vaped on no more than 10 days in their life. Former vapers were young people who had vaped on more than 10 days in their life, but who had not vaped in the past 30 days. Current vapers were young people who had vaped on more than 10 days in their life and who had vaped in the past 30 days.

3.4 Attempts to quit vaping

The ITC Youth survey asked past 30-day vapers if they had ever tried to quit vaping products. A little over a third of respondents had ever tried to quit vaping products (32.4%), with 61.4% not having ever tried to quit vaping products. There was little change since 2019 (Figure 4).

Figure 4. Ever tried to quit vaping products among young people aged 16 to 19 who have vaped in the past 30 days, England 2019 to 2021 (ITC, weighted data)



Notes: Unweighted base: 2019=368, 2020=536, 2021=567. Participants who had vaped in the past 30 days.

3.5 Effects of COVID-19 on vaping and smoking

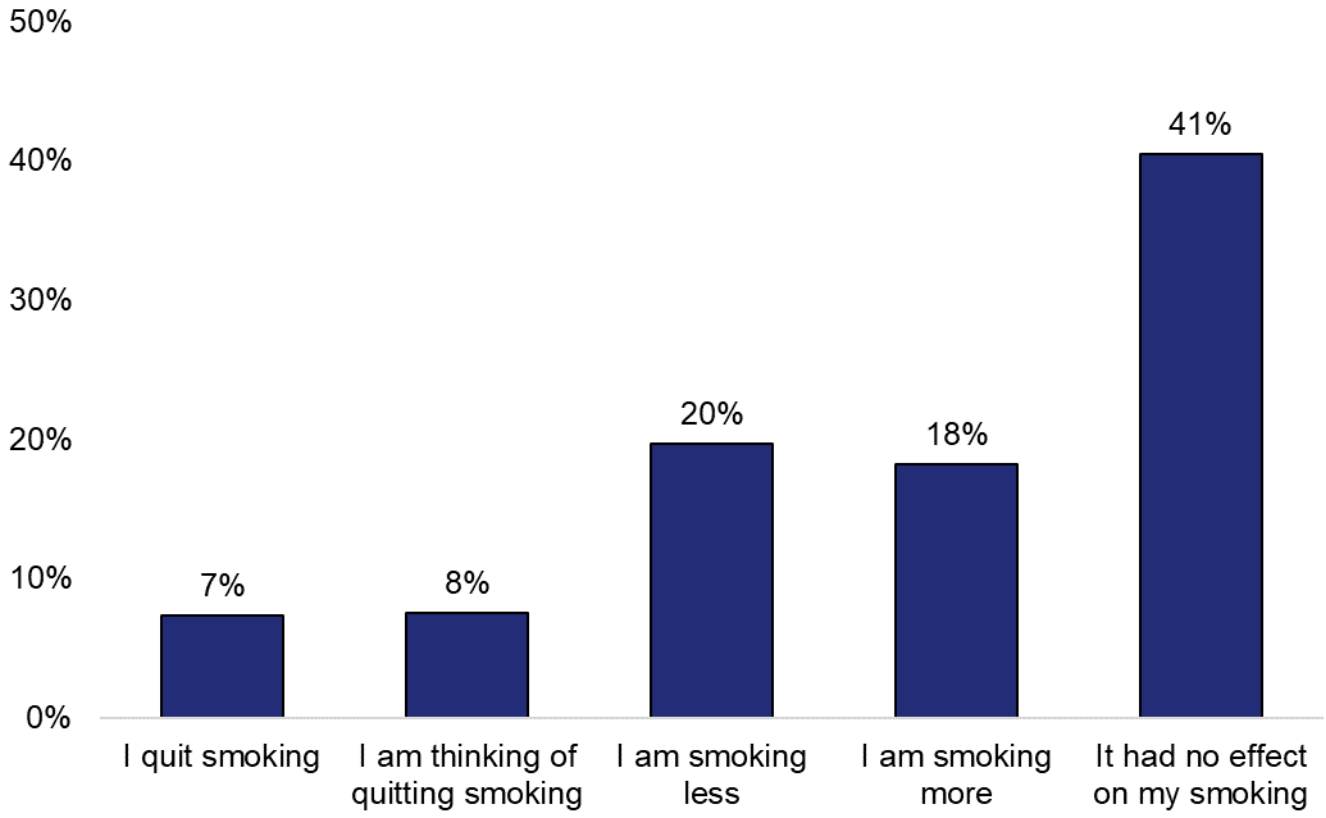
In response to the COVID-19 pandemic, schools were closed in England between the 4 January and 15 March 2021, and there were tight restrictions on social gatherings between the 4 January and the 19 May 2021.

International research has indicated COVID-19 has affected youth vaping and smoking behaviours (3, 4). A fall in vaping among youth in the US was reported to be associated with reduced access to retail environments, such as a change in opening hours or vaping products no longer being available (5, 6). Later in this chapter we discuss self-reported effects of COVID-19 on vaping and smoking from the ITC Youth survey in 2021.

According to 2021 ITC Youth survey data, just over half of young people who had smoked or vaped in the past 12 months reported that the COVID-19 outbreak had affected their vaping and smoking behaviours (Figure 6).

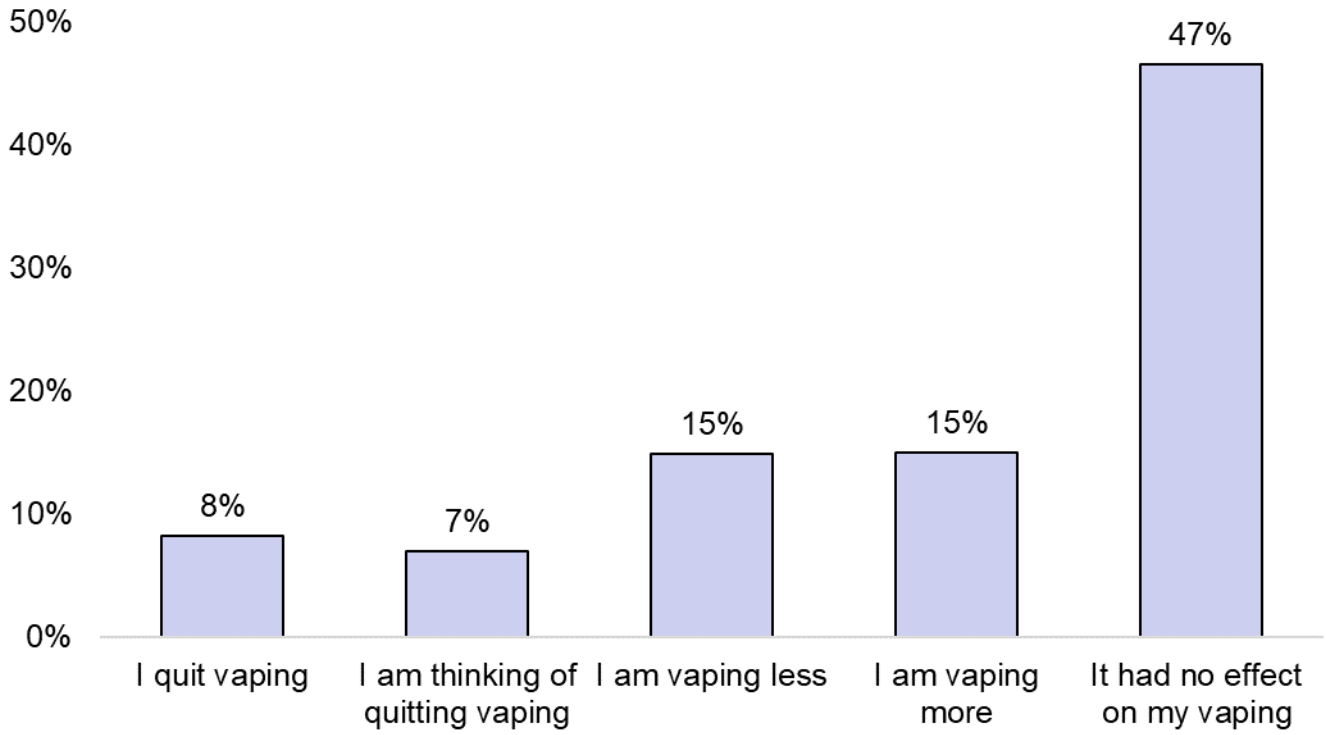
Seven percent of smokers and 8.0% of vapers reported quitting smoking and vaping respectively because of COVID-19. Twenty percent reported smoking less and 15.0% reported vaping less; and 8.0% of smokers and 7.0% of vapers reported thinking of quitting due to the COVID-19 pandemic. However, 18.0% reported smoking more and 15.0% reported vaping more. Just under half of past 12-month smokers and vapers reported no effect on their smoking (41.0%) or vaping (47.0%) respectively. Overall, the findings that a greater proportion of youth reported smoking or vaping less, or quitting smoking or vaping, than reported smoking or vaping more, could contribute to the slight increase in former smokers (from 0.8% to 1.7%) and former vapers (from 4.6% to 8.6%) observed between 2019 and 2021.

Figure 5. Self-reported effect of the coronavirus outbreak on smoking among young people aged 16 to 19 who smoked in the past 12 months, England 2021 (ITC Youth, weighted data)



Notes: Unweighted base N=1,235, participants who had smoked in the past 12 months. 6.0% (n=93) said they did not know or refused to answer, therefore percentages might not total 100%.

Figure 6. Self-reported effect of the coronavirus outbreak on vaping among young people aged 16 to 19 who vaped in the past 12 months, England 2021 (ITC Youth, weighted data)



Notes: Unweighted base N=1,330, participants who had vaped in the past 12 months. 8.0% (n=106) said they did not know or refused to answer, therefore percentages might not total 100%.

3.6 Reasons for vaping

The 2021 ASH-Y and ITC Youth surveys asked participants about reasons for vaping, but different groups of participants were asked these questions, and the reasons listed also differed. The ASH-Y survey asked all participants who had ever vaped, whereas the ITC survey asked participants who had vaped in the past 30 days. The ITC survey also differed from the ASH-Y survey because participants could choose multiple reasons for vaping from a list of 15, whereas the ASH-Y survey reported participants' single main reason for vaping from a list of 10.

In 2021, the most common reasons for vaping reported by young people were to 'give it a try' (2021 ASH-Y– 48.8%) or 'curiosity/to try something new' (ITC – 20.5%), and 'for the flavours' (ITC – 37.2%) or 'other people use them so I join in' (2021 ASH-Y– 16.6%) (Figure 7 and Figure 8).

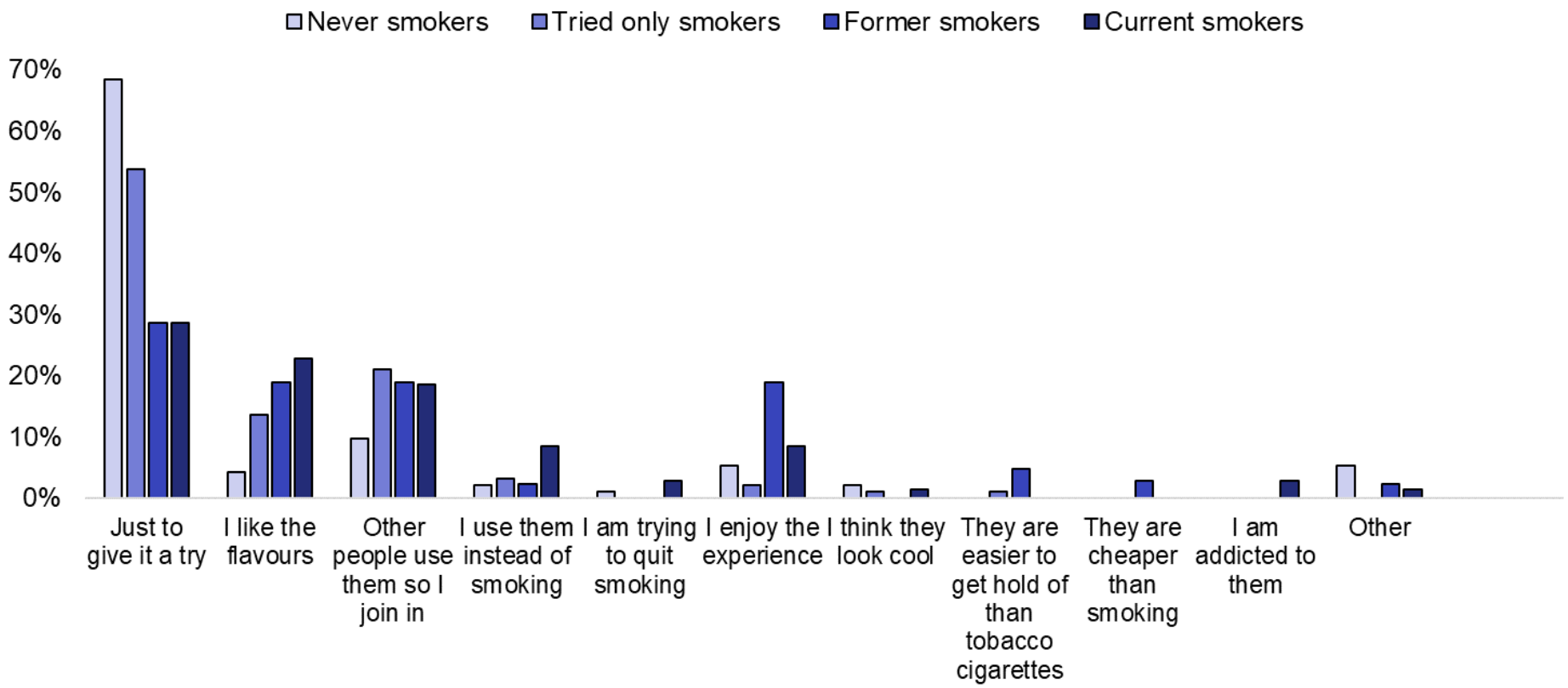
Reasons related to smoking prevention or cessation (for example ‘I use them instead of smoking’ or ‘I am trying to quit smoking’) were selected by 5% of ASH-Y participants overall. In the ITC Youth survey, 20.5% selected at least one reason related to quitting smoking, perhaps reflecting the older age range (16 to 19) in which smoking is more prevalent, and the option to choose multiple reasons for vaping in the ITC survey. In the ITC Youth survey, 29.2% of people who vaped in the past 30 days selected at least one reason related to reducing harm (for example ‘vaping may be less harmful to people around me than smoking’ or ‘vaping is less harmful to me than smoking’).

The reasons for vaping differed according to smoking status (Figure 7 and Figure 8). ASH-Y data indicate that high proportions of never smokers (68.5%) and those who had tried smoking only (53.7%) had vaped just to ‘give it a try’. This latter figure suggests that there may be a group of young people who experiment with both smoking and vaping but do not become regular users, although this cannot be tested with cross sectional data. Just over a quarter (28.6%) of current smokers also vaped to ‘give it a try’. Figure 7 also serves as a reminder that survey responses contain inconsistencies or inaccuracies as there was a small number of young people who responded that they were never smokers and were using vaping to quit smoking.

The ITC Youth data show that high proportions of former and current smokers vaped for smoking reduction or cessation reasons, including to cut down on or reduce the number of cigarettes they smoked (Figure 8). Among current smokers, 37.9% reported vaping to cut down on the number of cigarettes they smoked and 28.4% to help them quit.

Among former smokers, 19.3% reported vaping because it might be less harmful to people around them than smoking and 16.1% to help them maintain abstinence from cigarettes. The most common reason for vaping reported by 45.2% of former smokers in the ITC survey was because vaping may be less harmful for them than smoking.

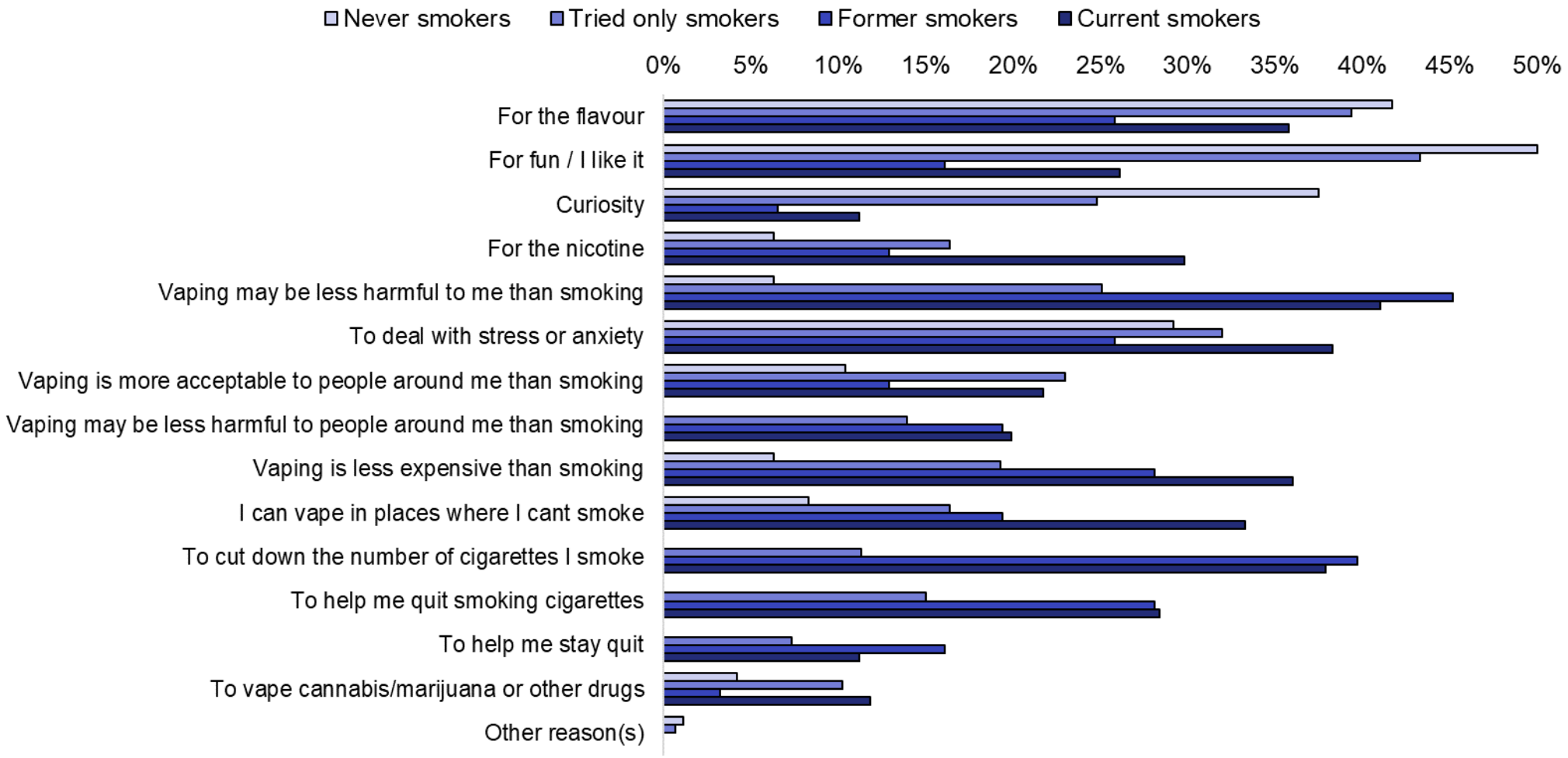
Figure 7. Main reason for vaping by smoking status among young people aged 11 to 18 who have ever vaped, England 2021 (ASH-Y, weighted data)



Notes: Unweighted base=337; Never smoker n=96, Tried only smokers n=108, Former smokers n=47, Current smokers n=84, Refused n=2. Participants could choose a single, main reason for vaping. Young people who have ever vaped comprised current, former and tried only vapers. Never smokers were young people who had never tried cigarettes. Tried only smokers were young people who had only ever tried smoking cigarettes once. Former smokers were young people who used to smoke sometimes but

who never smoked now. Current smokers were young people who smoked sometimes but less than weekly, as well as those who smoked more than once a week.

Figure 8. All reasons for vaping by smoking status among young people who vaped in the past 30 days, England 2021 (ITC Youth, weighted data)



Notes: Unweighted base N=558 Never smokers n=59, Tried only smoker n=302, Former smokers n=32, Current smokers n=165 Participants could choose multiple reasons. Young people who had vaped in the past 30 days. Never smokers were young people who had never tried cigarettes. Tried only smokers (referred to as ‘Experimental smokers’ in the ITC survey) were young people who had tried cigarettes, but who had not smoked more than 100 cigarettes in their life. Former smokers were young people who had smoked more than 100 cigarettes in their life, but who had not smoked in the past 30 days. Current smokers were young people who had smoked more than 100 cigarettes in their life and who had smoked in the past 30 days.

3.7 Order of first use of cigarettes and vaping products

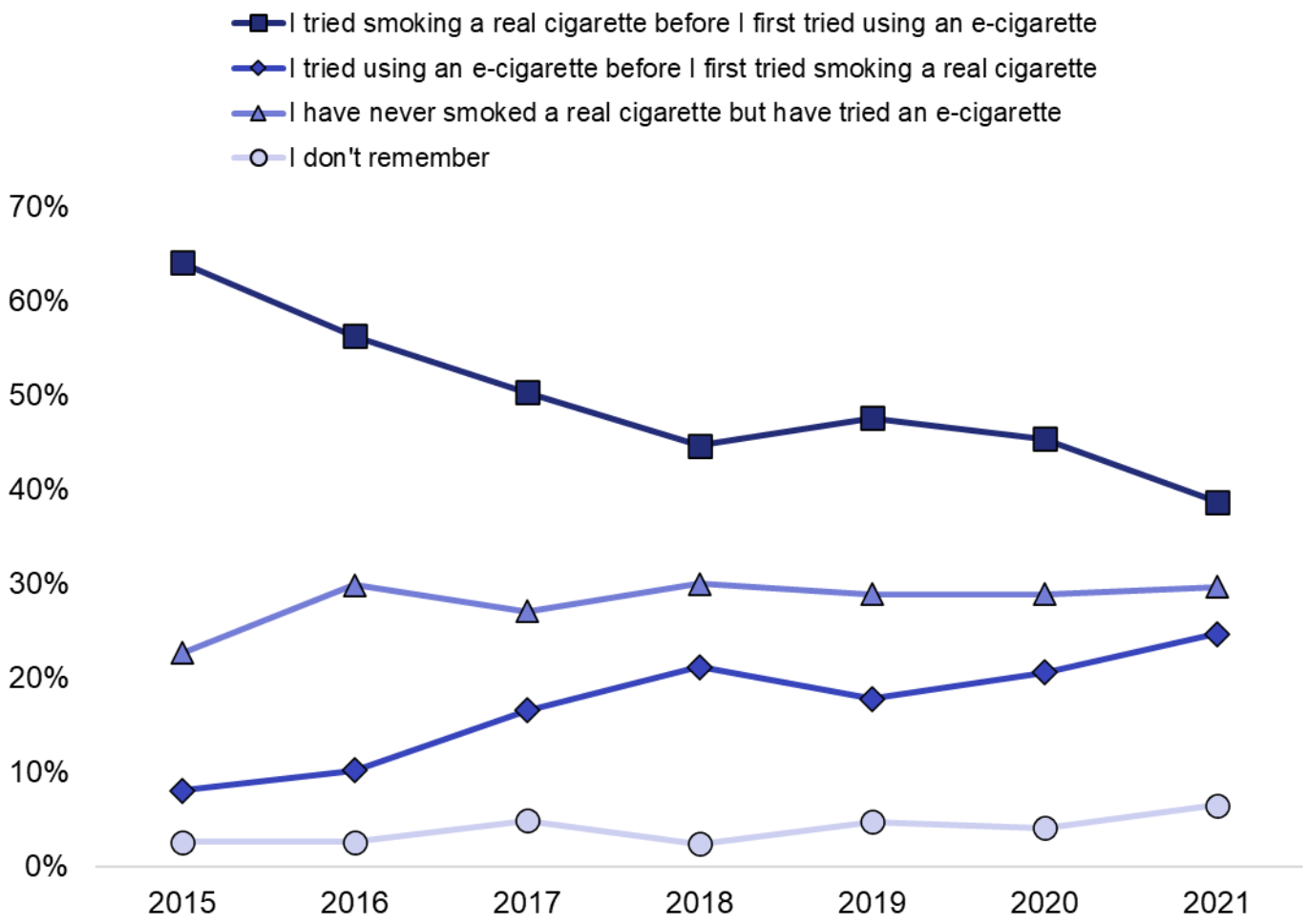
The ASH-Y survey participants have been asked to report the order in which they first tried cigarettes or vaping products since 2015, enabling changes to be tracked over time. The ITC Youth survey participants reported order of first use across a range of tobacco products, not limited to cigarettes and vaping products. To note, data presented here are repeated cross-sectional and cannot provide evidence for causal relationships.

Among young people who had ever vaped, according to the ASH-Y surveys, the order in which they first used cigarettes and vaping products appears to have changed in the past 7 years (Figure 9). From 2015 until 2018, a decreasing proportion of young people had tried smoking before vaping and an increasing proportion reported vaping before smoking. Since 2018, trends have remained relatively steady, with slightly fewer youth aged 11 to 18 years reporting smoking before vaping in 2021 (38.7%) compared to 2018 (44.7%). Trying vaping but never having smoked has remained at around 30.0% since 2016 (Figure 9).

The order of first use of vaping and smoking by socio-demographic characteristics is presented in table 5; however, conclusions should be considered tentative due to the small sample size. Smoking before vaping appears to be more common among 11 to 15 year olds (40.0%) and 18 year olds (41.9%), than among 16 to 17 year olds (35.9%). For social grade, 26.4% of people in groups ABC1 vaped before they smoked compared with 19.7% in groups C2DE; however, 39.4% of those in C2DE groups reported that they had ever vaped but never smoked, compared with 26.8% of young people from ABC1 groups. Males were more likely to vape before smoking (30.1%) than females (19.4%).

Among 16 to 19 year olds who had ever smoked, vaped, used heated tobacco products or any other nicotine or tobacco product, the ITC survey reported the type of product that users tried first; cigarettes were the most commonly used first (52.7%), followed by vaping products (33.4%), followed by other nicotine or tobacco products (9.0%), 4.9% did not know what product they used first.

Figure 9. Order of first use of cigarettes and vaping products among young people aged 11 to 18 who have ever vaped, England, 2015 to 2021 (ASH-Y, weighted data)



Notes: Unweighted bases: 2015=268; 2016=273; 2017=374; 2018=365; 2019=335; 2020=422 2021=337. Young people who have ever vaped comprised current, former and tried only vapers.

Table 5. Order of first use of cigarettes and vaping products among young people who have ever vaped by age, gender, region and social grade, England 2021 (ASH-Y, weighted data)

	Tried smoking before vaping % (n)	Tried vaping before smoking % (n)	Never smoked, but have vaped % (n)
Total	38.7 (117)	24.7 (74)	29.7 (90)
Age			
11 to 15	40.0 (34)	21.2 (18)	30.6 (26)
16 to 17	35.9 (47)	26.7 (35)	31.3 (41)
18	41.9 (36)	24.4 (21)	26.7 (23)
Gender			
Female	46.5 (67)	19.4 (28)	26.4 (38)
Male	31.4 (49)	30.1 (47)	32.7 (51)
Region			
North	37.7 (29)	27.3 (21)	32.5 (25)
Midlands	30.2 (16)	22.6 (12)	35.8 (19)
South	41.9 (72)	23.8 (41)	26.7 (46)
Social grade			
ABC1	39.0 (90)	26.4 (61)	26.8 (62)
C2DE	36.6 (26)	19.7 (14)	39.4 (28)

Notes: Unweighted base=337. Young people who have ever vaped comprised current, former and tried only vapers. 4.1% (n=19) said they did not remember which product they tried first, therefore percentages might not total 100.

3.8 Vaping products

In 2021, rechargeable models that have a tank that you fill with liquid (tank models) were the most popular vaping product type among ASH-Y and ITC Youth survey participants, with 41.0% of ASH-Y 11 to 18 year- olds who currently vaped using this type of product, and 67.7% of ITC Youth 16 to 19 year old past 30-day vapers using a tank model. The second most common product type was rechargeable products that use cartridges (ASH-Y: 36.4%; ITC Youth: 31.2%). Disposable vaping products were used by 7.8% of ASH-Y current vapers and 10.1% of ITC Youth past 30 day vapers in 2021 (Figure 10 and Figure 11). ITC Youth participants could choose more than one model, with most reporting current use of one type of model (86.9%) and 10.5% reporting current use of multiple models.

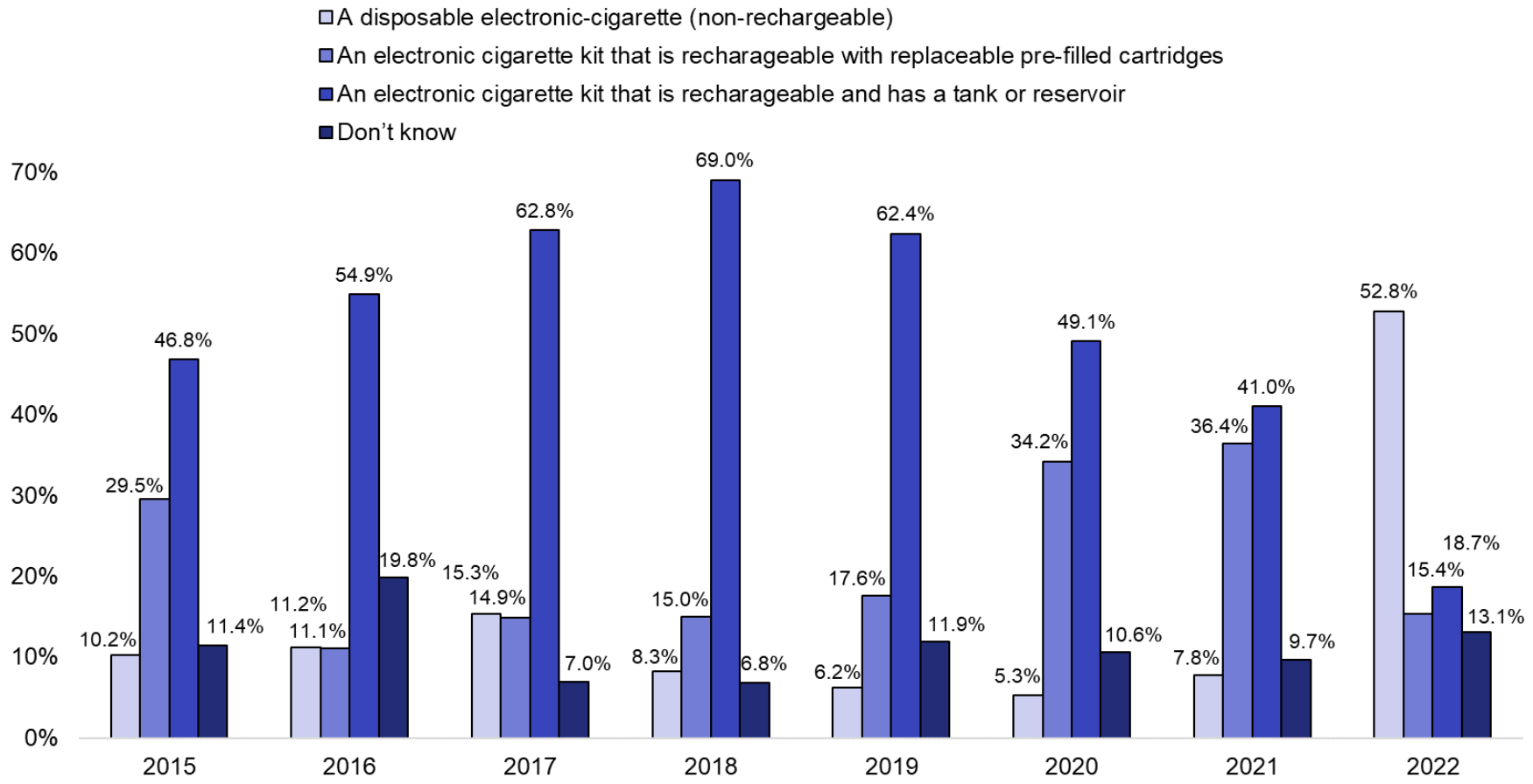
However, in 2022, the picture had changed considerably (Figure 10) with the ASH-Y data showing a substantial increase in youth reporting the use of disposable products between

2021 (7.8%) and 2022 (52.8%). This is likely due to the introduction of a new generation of disposable products available in a range of colours and flavours which are often displayed at the point of sale.

The popularity of tank models among youth in ASH-Y declined between 2018 and 2022 (Figure 10) likely initially because of the increasing popularity of cartridge models between 2018 (15.0%) and 2021 (36.4%). This trend was probably driven by pod models, but also possibly the introduction to the market of refillable cartridges. The popularity of tank and cartridge models was much lower in 2022 as disposables became the most popular type of vaping product used.

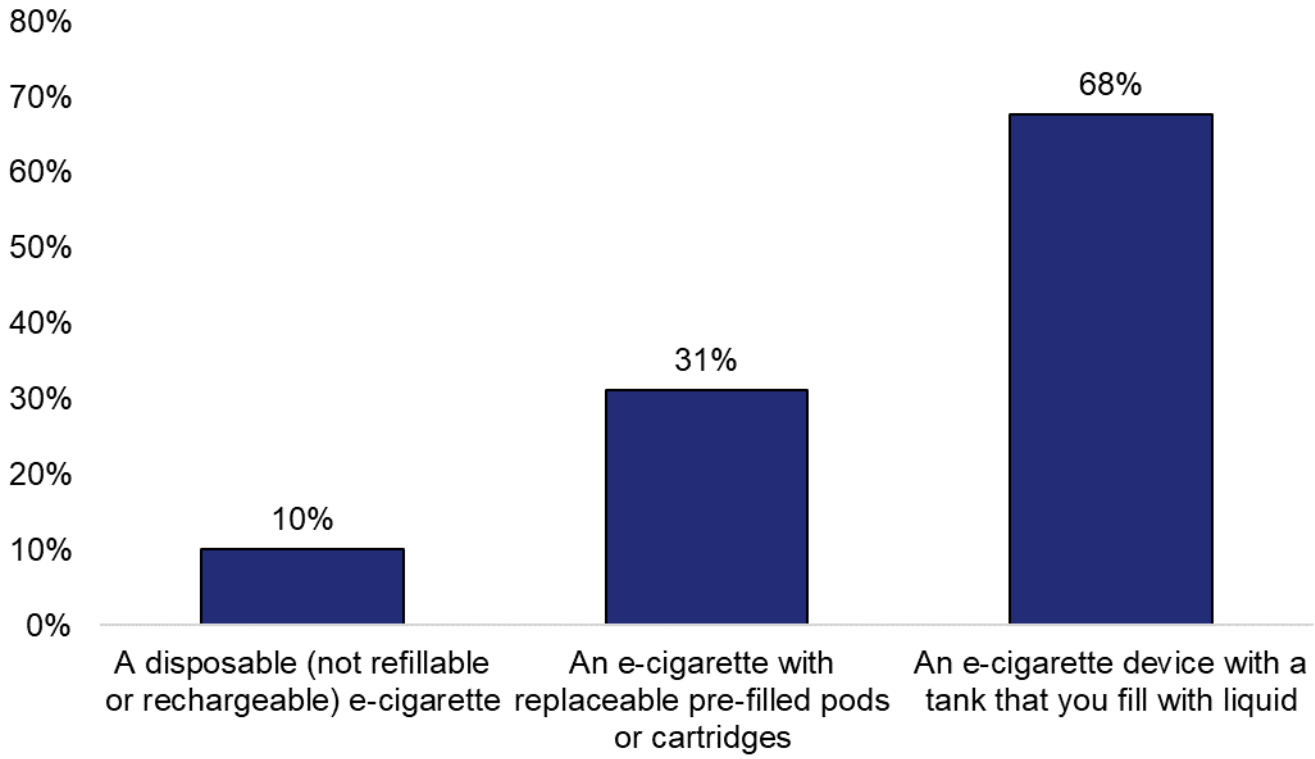
In 2021, the ITC Youth survey reported that the most popular brands were Smok (22.7%), which includes tank, refillable cartridge and disposable models; JUUL (18.3%), a brand that sells only cartridge models; Blu (10.1%) which sells tank and cartridge models; 88 Vape (7.7%) which sells tank and refillable cartridge models and Vype (5.2%), which sells tank and cartridge models.

Figure 10. Type of vaping product used by young people aged 11 to 18 who currently vape, England 2015 to 2022 (ASH-Y, weighted)



Notes: Unweighted bases; 2015=52; 2016=57; 2017=82; 2018=77; 2019=106; 2020=117; 2021=99; 2022=233. Current vapers were young people who vaped at least monthly, weighted data.

Figure 11. Type(s) of vaping product used most often by young people aged 16 to 19 who had vaped in the past 30 days, England 2021 (ITC Youth, weighted data)



Notes: Unweighted base N=567. Participants who had vaped in the past 30 days.

3.9 Flavours

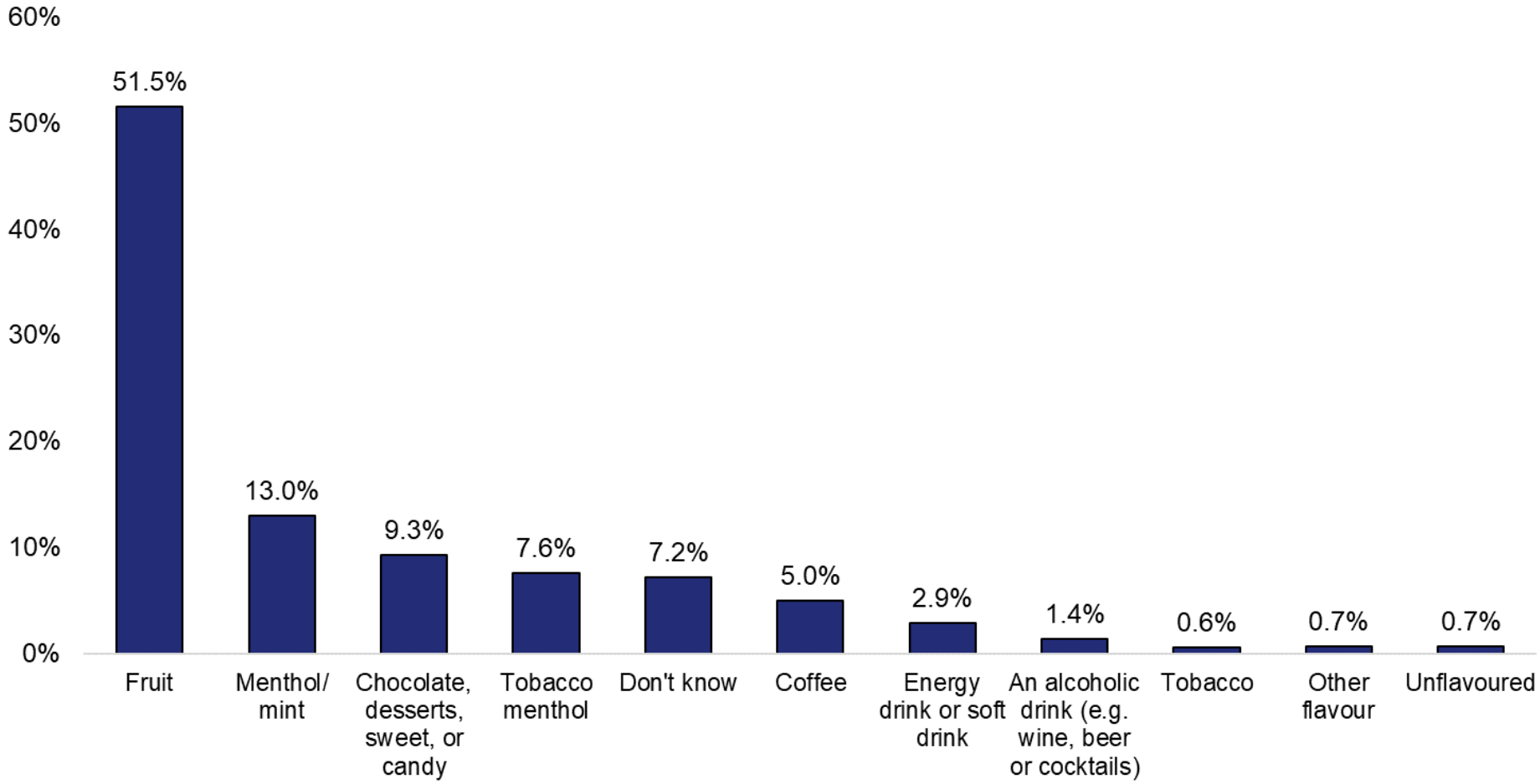
The 2021 ASH-Y survey assessed the flavour most often used by young people who currently vaped, while the ITC Youth survey collected data on the flavour(s) used most often among young people who had vaped in the past 30 days. Participants could only choose one option for ASH-Y but could choose multiple options for ITC Youth.

The 2021 ASH-Y data estimated that fruit flavoured vaping products were used by 51.5% of 11 to 18 year olds who vaped (Figure 12) followed by menthol or mint (13.0%) and chocolate, desserts, sweet or candy flavours (9.3%). These flavour patterns are very similar to the ASH-Y 2020 data used in our 2021 report (1), where fruit was the most commonly used (46.3%), followed by menthol or mint (18.1%) and chocolate, sweets or candy (8.5%).

The ITC Youth data reports similar flavours that were most often used among 16 to 19 year olds who vaped in the last 30 days, with 63.7% using fruit flavours, 27.7% using menthol or mint flavours and 10.6% using chocolate, desserts, sweet or candy flavours

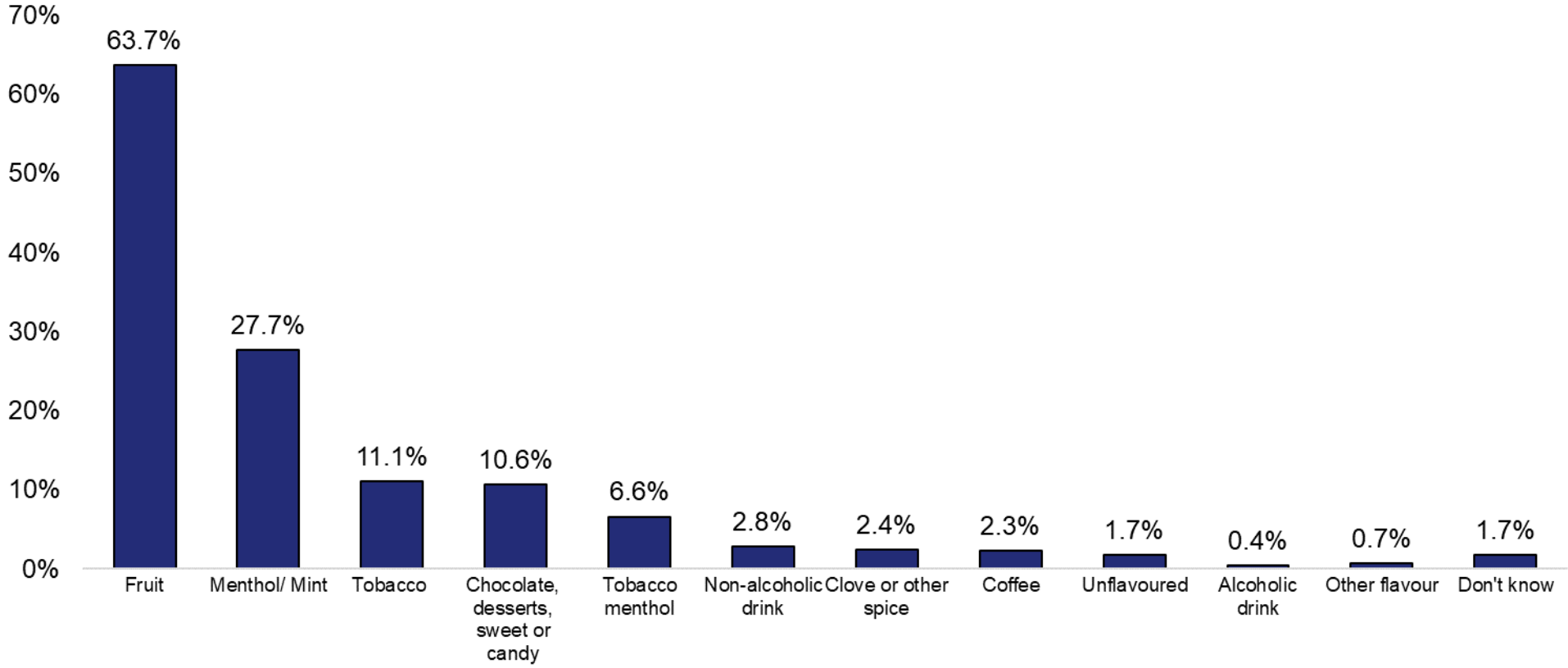
(Figure 13). The ITC Youth survey also indicates that 11.1% used tobacco flavours (0.6% in ASH-Y). All other flavours, across both surveys, were used by less than 10% of included participants. Again, these flavour patterns are very similar to the ITC Youth 2019 data used in our 2021 report (1), where fruit was the most commonly used (67.7%), followed by menthol or mint (18.3%), chocolate, sweets or candy (13.5%) and tobacco (10.3%).

Figure 12. Vaping flavour used most often among young people aged 11 to 18 who currently vape, England 2021 (ASH-Y, weighted data)



Notes: Unweighted base=99. Current vapers were young people who vaped at least monthly.

Figure 13. Vaping flavours used most often among young people aged 16 to 19 who vaped in the past 30 days, England 2021 (ITC Youth, weighted data)



Notes: Participants who had vaped in the past 30 days and had reported ever using the flavour. Unweighted bases therefore differed by flavour.

3.10 Nicotine

In the 2021 ASH-Y survey, 34.2 % of current and former vapers aged 11 to 18 reported they used vaping products that always contained nicotine, 35.5% said their products sometimes contained nicotine, 20.4% said their products never contained nicotine and 9.9% said they did not know (Figure 14).

In the ITC Youth survey, 68.9% of people aged 16 to 19 who had vaped in the past 30 days and had ever used vaping products with nicotine said their current products contained nicotine, 14.4% said some of their products contained nicotine, 12.2% said their current product did not contain nicotine and the remaining 4.5% did not know (Figure 15).

The proportion of vapers who currently used nicotine containing vaping products was larger in the ITC Youth survey (68.9%). This was likely due to the fact that this question was only asked to those who indicated ever vaping nicotine and the different age range.

Using ITC Youth data, when those who had vaped in the past 30 days and reported they had ever vaped nicotine were asked about their current nicotine strength, 53.7% said they used less than 2% (20 milligrams per millilitre (mg/mL)), 17.2% said they used between 2% and 4.9% strength (20 to 49mg/mL), 5.6% said they used 5% or more (≥ 50mg/mL), and 16.2% said they currently used no or 0% nicotine, and 7.3% saying they didn't know. Strengths over 2% (20mg/mL) are illegal to sell in the UK. From the response options available, we are unable to discern exactly what proportion of participants used 2% strength and what proportion used over 2% strength. Nevertheless, at least 5.6% of past 30 day vapers reported they used a nicotine concentration that is illegal to sell. Additionally, 7.3% of past 30-day vapers did not know the nicotine strength of the liquid they used. Finally, responses were based on self-reported nicotine use and it is possible that there is limited knowledge surrounding nicotine strength of products among young people so these may be unreliable indicators of nicotine consumption among this age group (Figure 16).

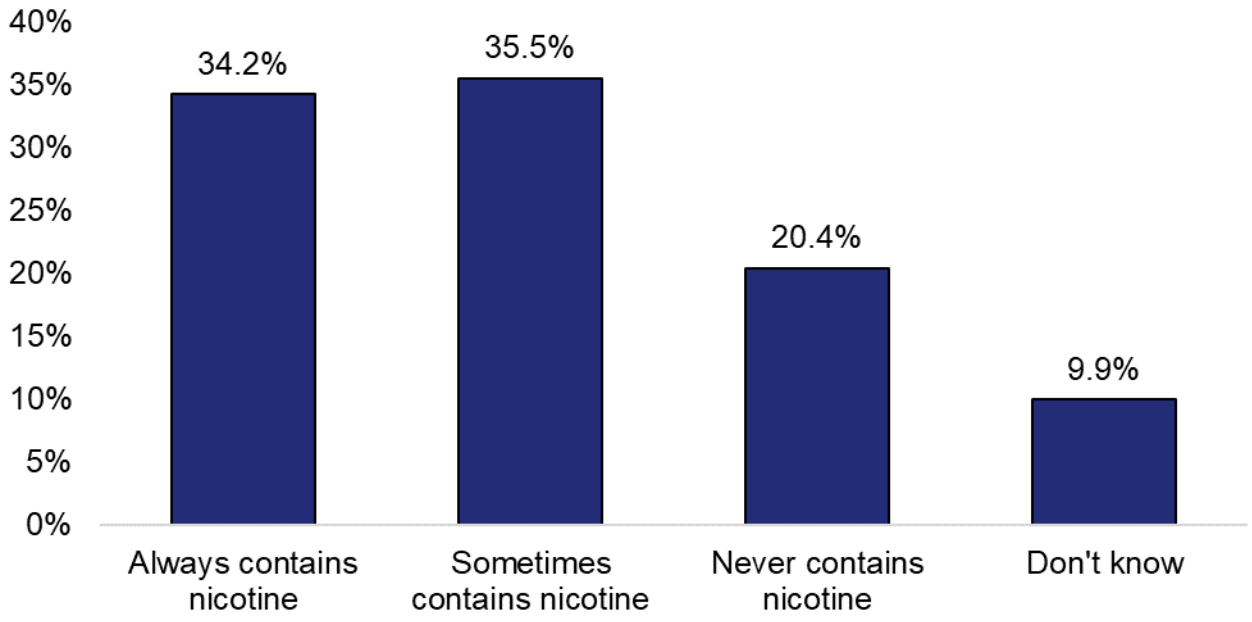
The ITC Youth data indicated that just over half (53.1%) of young people who vaped in the past 30 days and were aware of nicotine salts, currently used nicotine salts, 40.4% said they did not currently use nicotine salts and 6.5% did not know (Figure 17). Participants in the ASH-Y survey were not asked about nicotine salts.

Among young people aged 16-19 who reported using nicotine e-liquids of 20 to 49mg/mL nicotine strength, 10.8% reported using disposable products, 28.1% reported using cartridge products and 75.0% reported using tank products. For those using 50mg/mL or more, 4.8% reported using disposable products, 28.6% reported using cartridge products and 66.7% reported using tank products (table 6), although small sample sizes were low in some cells.

Among young people aged 16-19 who reported using non-nicotine e-liquids, e-liquids with less than 20mg/mL and with 20 to 49mg/mL nicotine strength, vaping products were most commonly reported to be bought from vape shops. Participants using vaping liquids with 50mg/mL or more nicotine most commonly reported buying these products online (36.4%) (table 6). For those who did not know the strength of the nicotine they used, vaping products were most commonly reported to be given to them (33.3%).

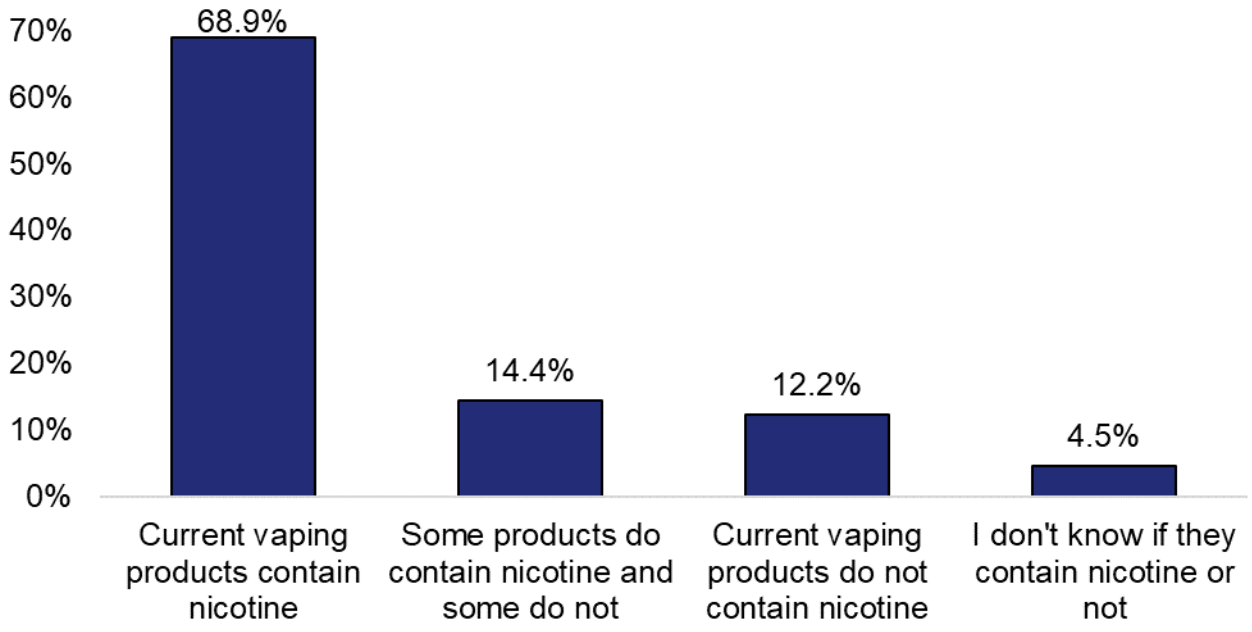
Overall, in the ITC Youth data, there was higher awareness of the inclusion of nicotine and type of nicotine in vaping products, and fewer don't know responses in 2021 compared to 2019 data reported in our 2021 report.

Figure 14. Use of nicotine vaping products among young people aged 11 to 18 who are current and former vapers, England 2021 (ASH-Y)



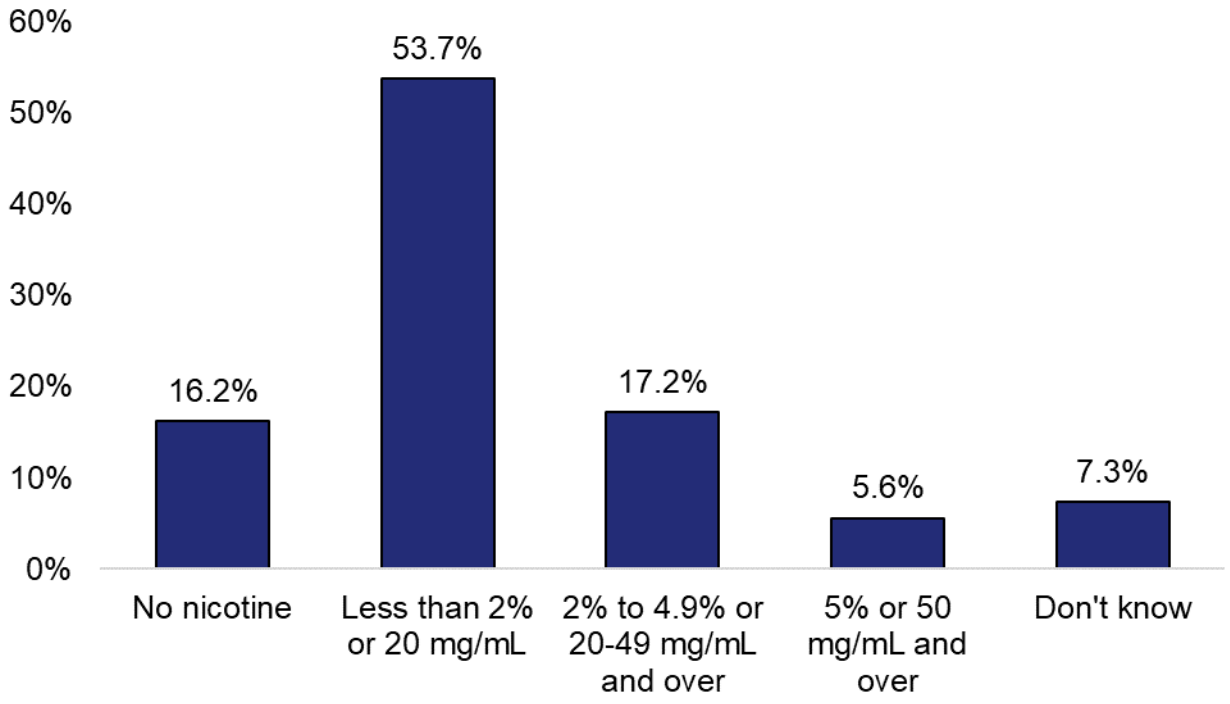
Notes: ASH-Y: Unweighted base=125. Current vapers were young people who vaped at least monthly. Former vapers were young people who used vaping products in the past but who no longer do so.

Figure 15. Use of nicotine vaping products among young people aged 16 to 19 who have vaped in the past 30 days and had used vaping products with nicotine, England 2021 (ITC Youth)



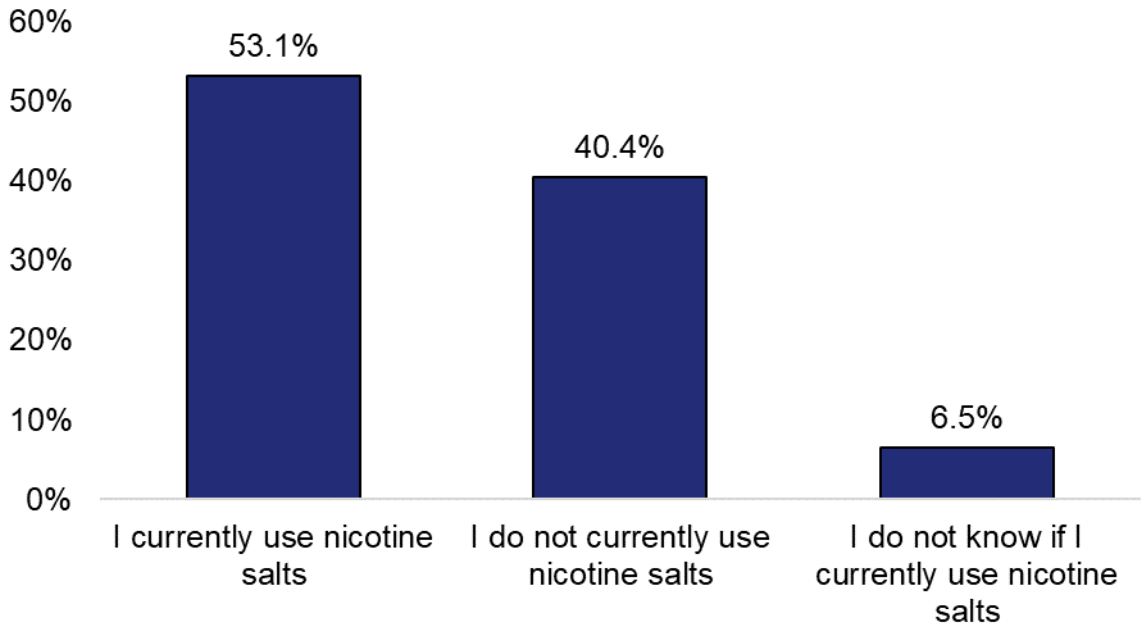
Notes: Unweighted base=405. Participants who have vaped in the past 30 days and who have every vaped nicotine.

Figure 16. Strength of nicotine in vaping liquids among young people who have vaped in the past 30 days and currently use vaping products with nicotine, England 2021 (ITC Youth, weighted data)



Notes: Unweighted base=342. Participants who have vaped in the past 30 days and currently vape nicotine.

Figure 17. Use of nicotine salts among young people aged 16 to 19 who have vaped in the past 30 days and have used vaping products with nicotine, England 2021 (ITC Youth, weighted data)



Notes: Unweighted base=171. Participants who have vaped in the past 30 days and currently vape nicotine and have heard of nicotine salts.

Table 6. Strength of current nicotine used among current vapers aged 16 to 19 by current product and source of products, England 2021 (ITC Youth, weighted data)

	No nicotine (0 mg/mL) %(n)	< 20 mg/mL %(n)	≥ 20 mg/mL < 50 mg/mL %(n)	≥ 50 mg/mL %(n)	I don't know %(n)
Types of vaping product used most often in the past 30 days					
Disposable	3.3 (2)	8.4 (17)	10.8 (7)	4.8 (1)	7.4 (2)
Cartridge	18.0 (11)	41.3 (83)	28.1 (18)	28.6 (6)	22.2 (6)
Tank	80.3 (49)	64.4 (130)	75.0 (48)	66.7 (14)	77.8 (21)
Sources of product in past 30 days					
Bought from a store	37.7 (23)	54.5 (110)	47.7 (31)	28.6 (6)	29.6 (8)
Bought online	23.3 (14)	32.3 (65)	25.0 (16)	36.4 (8)	3.7 (1)
Bought them from a person	9.8 (6)	9.0 (18)	12.3 (8)	23.8 (5)	7.4 (2)
Gave someone money to buy them for me	5.0 (3)	7.5 (15)	18.8 (12)	9.5 (2)	22.2 (6)
Someone gave them to me	31.1 (19)	21.9 (44)	35.4 (23)	28.6 (6)	33.3 (9)
Free sample	3.3 (2)	5.4 (11)	4.7 (3)	4.8 (1)	0
Took them	0	2.5 (5)	1.5 (1)	0	3.7 (1)

Notes: Unweighted base=342. Participants who had vaped in the past 30 days and currently vape nicotine. Participants could choose multiple response options.

3.11 Perceived addiction and urges to vape

In the 2021 ITC survey, youth who had smoked or vaped in the past 30 days were asked if they considered themselves addicted. Among current vapers, half (52.5%) reported they were addicted ('yes, a little addicted' or 'yes, very addicted') to using vaping products, just under half (42.8%) reported they were 'not at all' addicted to vaping products, 4.7% reported 'don't know' (Figure 18).

The proportion of ITC youth who had vaped in the past 30 days and considered themselves a little or very addicted to vaping products was higher among current (63.6%)

and former (74.2%) smokers than those who had never smoked (20.0%). The proportion who considered themselves a little or very addicted was also higher among participants aged 19 (61.5%), than those aged 18 (52.6%), 17 (44.3%), or 16 (51.7%). Moreover, feeling a little or very addicted was also more common among male participants (56.1%) than females (48.7%; table 7).

Among current smokers, the majority considered themselves a little or very addicted to cigarettes (83.0%), with few participants reporting that they were ‘not at all’ addicted to cigarettes (14.5%), 2.5% reported ‘don’t know’ (Figure 18).

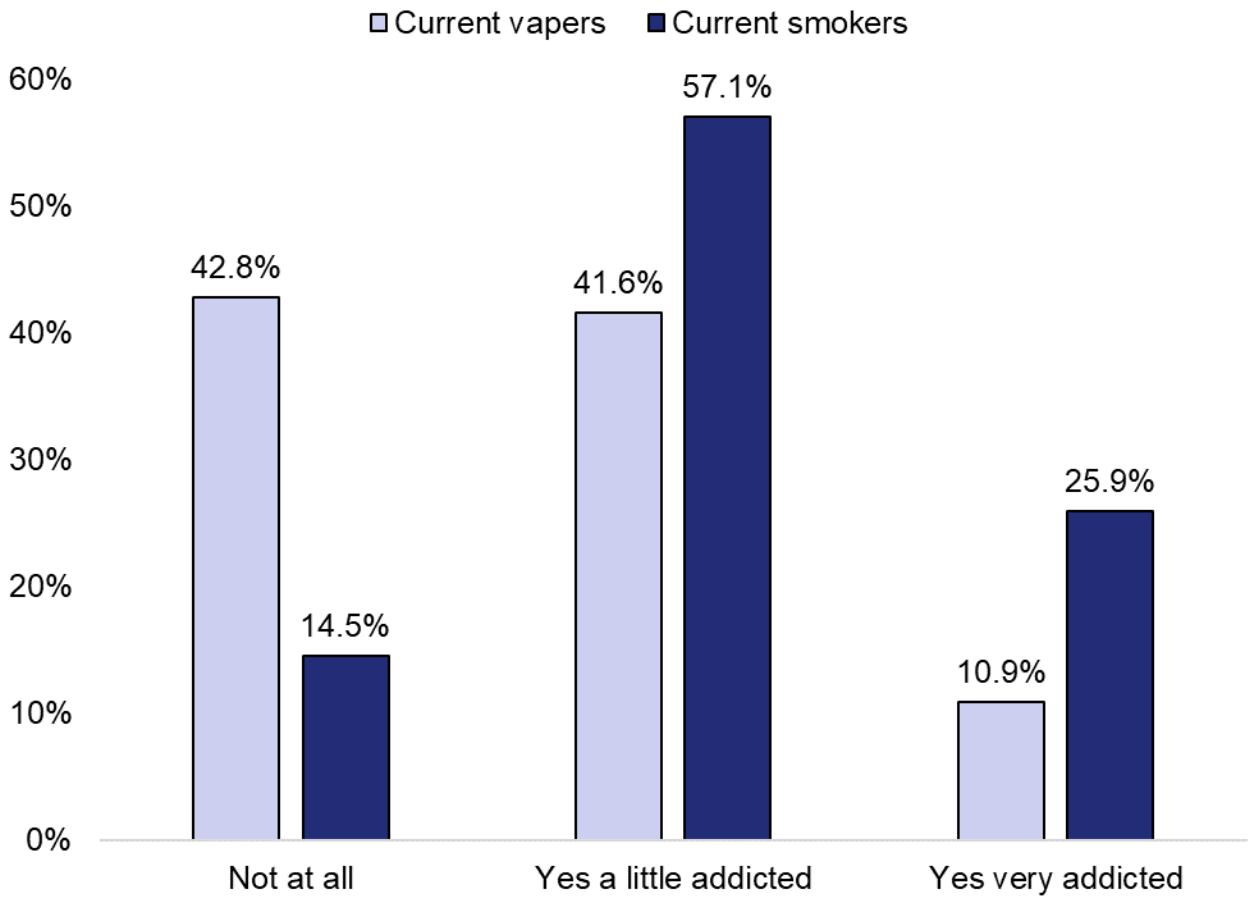
Another indicator of addiction is the strength and frequency of urges to vape. The ITC survey collected data on the frequency of urges to vape and smoke (Figure 19), and the 2021 ASH-Y survey collected data on strength of urges to vape and smoke (Figure 20).

Among current vapers in the ITC Youth survey, 16.8% never had urges to vape. Just over a third (34.7%) had urges weekly or less than weekly and 44.5% had urges almost daily or more than daily (Figure 1). In comparison, urges to smoke appeared to be more frequent among current smokers with only 4.7% reporting never having urges to smoke, 27.8% reporting urges weekly or less than weekly, and 66.6% reporting urges to smoke daily or multiple times a day.

In the ASH-Y survey, 4 in 10 (41.5%), of 11 to 18 year olds who currently vaped said they did not feel any urges to vape at all with a further 35.0% saying they felt slight or moderate urges and 23.5% reporting strong, very strong or extremely strong urges to vape. By contrast, 24.3% of current smokers reported no urge to smoke, with 44.2% reporting slight or moderate urges and 31.4% reporting strong, very strong or extremely strong urges to smoke.

Although the 2 surveys used different measures of addiction and sampled different populations, there is a common theme that those who smoked reported experiencing higher levels of addiction, urges to smoke and frequency of urges than those who vaped.

Figure 18. Frequency of considering oneself addicted to vaping among current vapers and considering oneself addicted to smoking among current smokers aged 16 to 19, England 2021 (ITC Youth, weighted data)



Notes: Unweighted bases Vaping=392; smoking=349. Current vapers were young people who had vaped on more than 10 days in their life and who had vaped in the past 30 days. Current smokers were young people who had had smoked more than 100 cigarettes in their life and had smoked in the past 30 days. 19 current vapers (4.7%) and 9 (2.5%) current smokers reported they did not know or refused to answer, therefore percentages may not total 100.

Table 7. Frequency of considering oneself addicted to vaping among current vapers aged 16 to 19, England 2021 (ITC Youth, weighted data)

	Not at all % (n)	Yes, a little or very addicted % (n)
Total	42.8 (168)	52.5 (206)
Age		
16	46.6 (27)	51.7 (30)
17	49.1 (52)	44.3 (47)
18	43.1 (59)	52.6 (72)
19	34.1 (31)	61.5 (56)
Gender		
Female	46.1 (89)	48.7 (94)
Male	39.9 (79)	56.1 (111)
Region		
North	41.6 (57)	54.0 (74)
Midlands	37.7 (26)	55.1 (38)
South	45.5 (85)	50.3 (94)
Ethnicity		
White	42.2 (137)	54.5 (177)
Black and minority ethnic groups	46.9 (30)	40.6 (26)
Smoking status		
Never smoked	70.0 (14)	20.0 (4)
Tried only	50.0 (100)	44.0 (88)
Former smoker	25.8 (8)	74.2 (23)
Current smoker	33.3 (44)	63.6 (84)

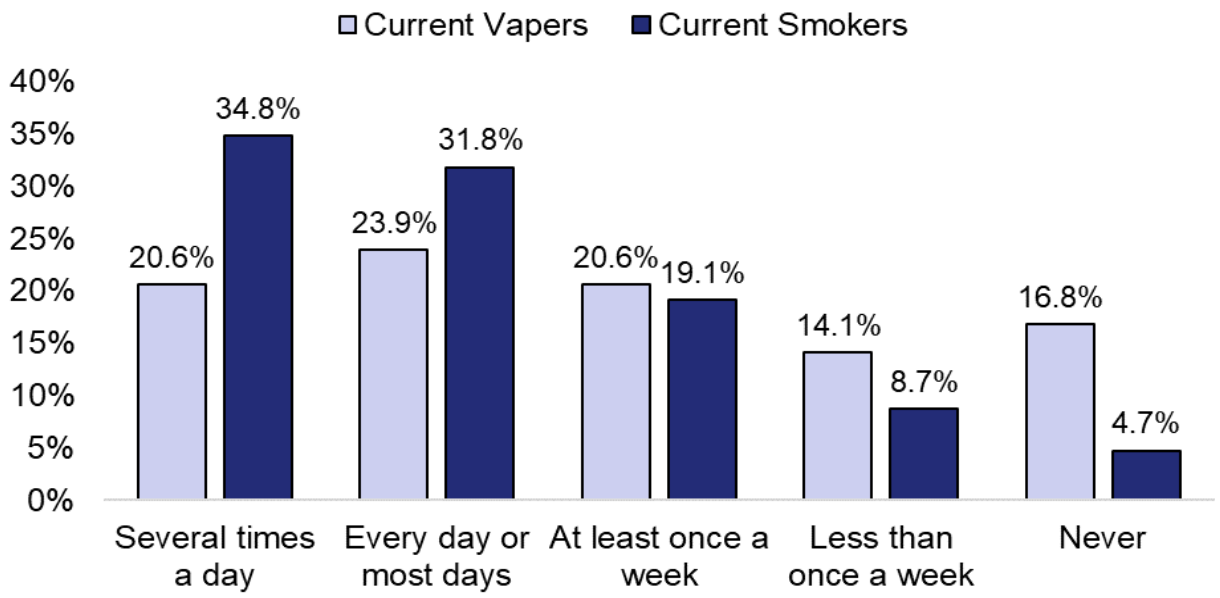
Notes: Unweighted base=392.

Current vapers were young people who had vaped on more than 10 days in their life and who had vaped in the past 30 days.

Never smokers were young people who had never tried cigarettes. Tried only smokers (referred to as ‘Experimental smokers’ in the ITC survey) were young people who had tried cigarettes, but who had not smoked more than 100 cigarettes in their life. Former smokers were young people who had smoked more than 100 cigarettes in their life, but who had not smoked in the past 30 days. Current smokers were young people who had smoked more than 100 cigarettes in their life and who had smoked in the past 30 days.

4.7% (n=19) said they did not know or refused to answer, therefore percentages might not total 100.

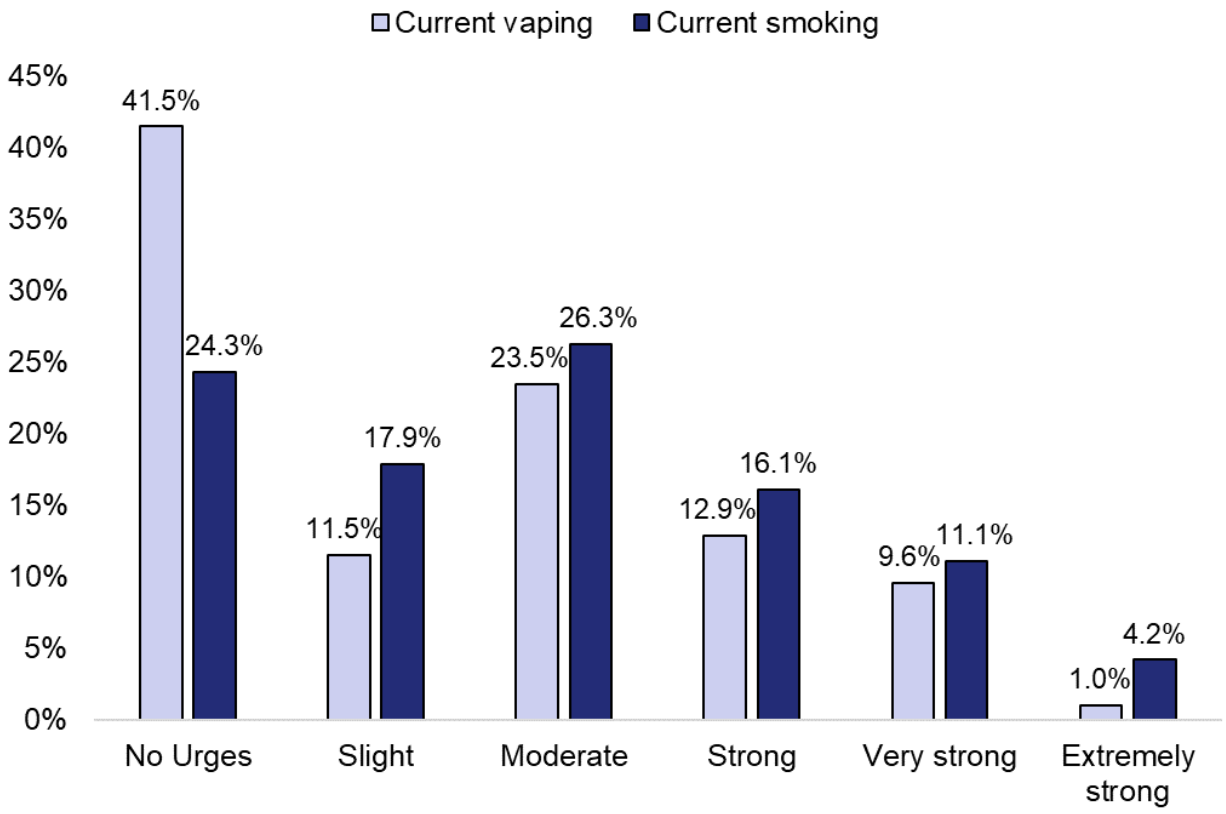
Figure 19. Frequency of urges to smoke or vape among young people aged 16 to 19 who currently smoke or had vaped in the past 30 days, England 2021 (ITC Youth, weighted data)



Notes: Unweighted bases Vaping=392; smoking=349. Current vapers were young people who had vaped on more than 10 days in their life and who had vaped in the past 30 days. Current smokers were young people who had had smoked more than 100 cigarettes in their life and had smoked in the past 30 days.

16 current vapers (4.0%) and 3 (0.9%) current smokers reported they did not t know or refused to answer, therefore percentages may not total 100.

Figure 20. Strength of urges to vape among current vapers and strength of urges to smoke among current smokers aged 11 to 18, England 2021 (ASH-Y, weighted data)



Notes: Unweighted bases Vaping=99; Smoking=105. Current vapers were young people who vaped at least monthly. Current smokers were young people who smoked sometimes but less than weekly, as well as those who smoked more than once a week.

3.12 Source, place of purchase and ownership

It is illegal in the UK to sell tobacco or vaping products to under-18s, and for adults to buy tobacco and vaping products on behalf of someone under the age of 18. Therefore, in this section we limit data to under 18 year olds.

For 2021 ASH-Y participants aged 11 to 17 (Figure 21), all participants who vaped at least monthly could provide one or more answers regarding where they get their vaping products from. Similarly, all current smokers could provide one or more answers regarding where they get their tobacco cigarettes from.

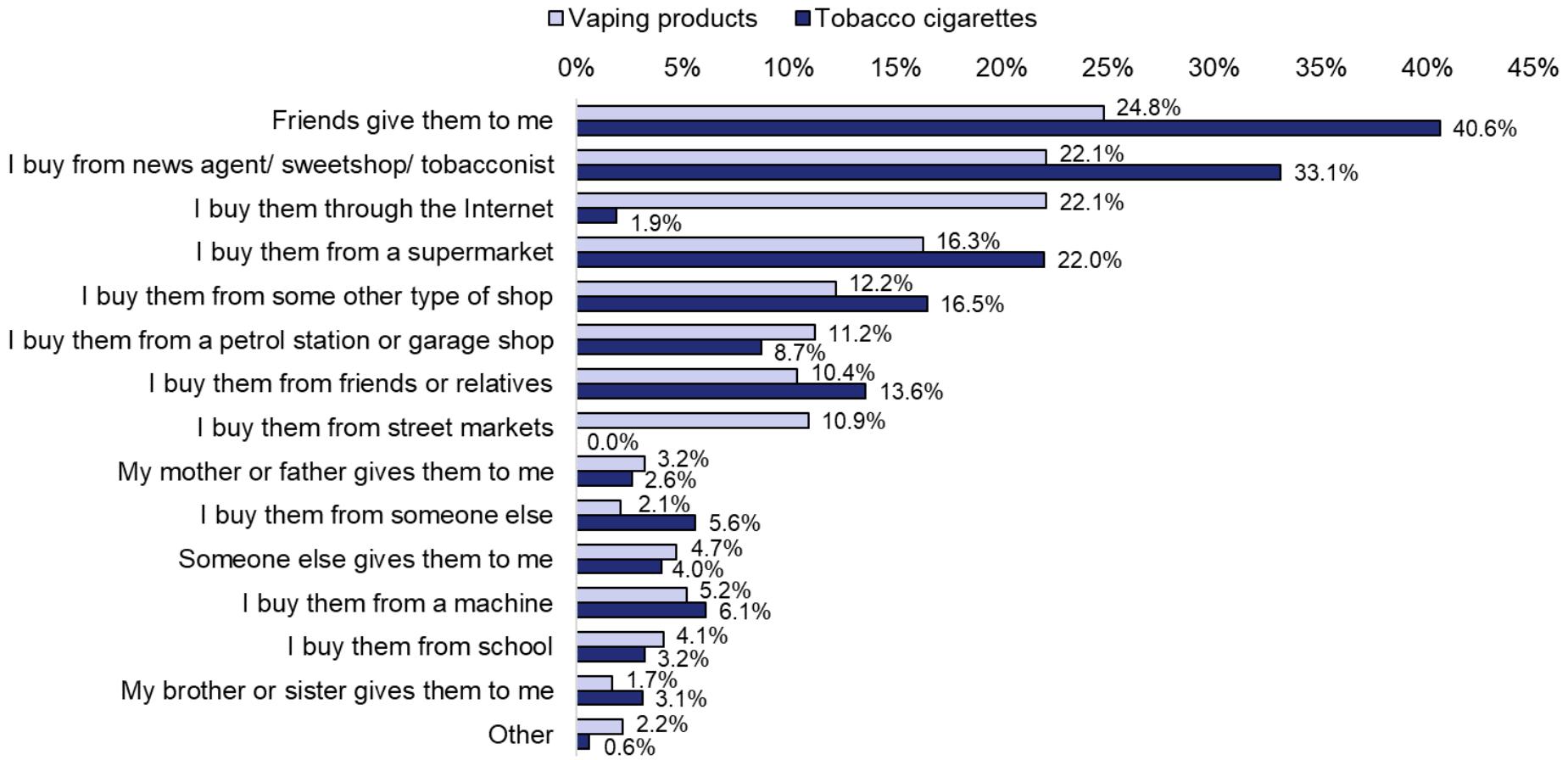
Under 18 year olds who were current vapers reported they obtained their products from several sources; just under a quarter (24.8%) reported being given their products by friends, 22.1% reported buying from newsagents, and 22.1% from the internet. The

proportion reporting buying from the internet was substantially higher in 2021 compared to 12.4% in 2020, possibly as the internet may have been the only source of products as a result of shop closures during the COVID-19 lockdowns. Just 3.2% said that their parents gave vaping products to them. Overall, although the sale of vaping products to under 18s is illegal, 7 out of 10 of the most popular sources of vaping products for underage youth were for purchases—from physical or online shops.

Similar to vaping, among current smokers under 18, many reported that friends give cigarettes to them (40.6%). Also, many reported buying cigarettes from shops, such as supermarkets (22.0%) or from newsagents (33.1%).

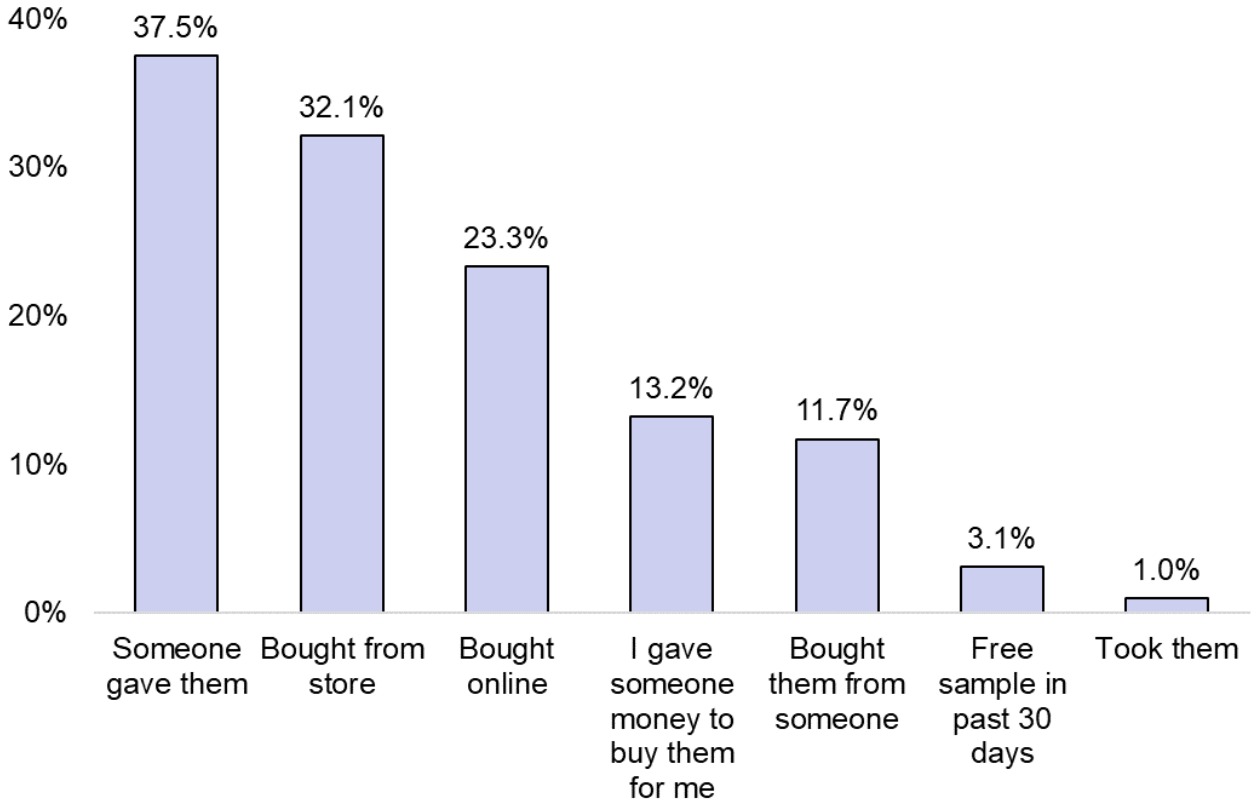
Using 2021 ITC-Youth data for under 18 year olds, among those aged 16 to 17 years who had vaped in the past 30 days, the most common source of vaping products was to be given them by someone (37.5%). However, similar to ASH-Y, purchase of vaping products was also common among those under 18, with over a third reporting purchasing products from a store (32.1%) and almost a quarter reporting purchasing products online (23.3%). Youth also reported giving someone else money to purchase products for them (13.2%), and purchasing them from someone else (11.7%). Some youth also reported being given a free sample of vaping products in the past 30 days (3.1%, Figure 22.).

Figure 21. Sources of vaping products and tobacco cigarettes used by current vapers and current smokers aged 11 to 17, England 2021 (ASH-Y, weighted data)



Notes: Unweighted bases; current vapers=66, current smokers=66. Participants could choose multiple response options. Current vapers were young people who vaped at least monthly. Current smokers were young people who smoked sometimes but less than weekly, as well as those who smoked more than once a week. Sources denoting that vaping products or tobacco cigarettes have been given to (rather than bought by) young people are in striped bars.

Figure 22. Sources of vaping products used by past 30 day vapers aged 16 to 17, England 2021 (ITC Youth, weighted data)



Notes: Unweighted base N=567. Participants who had vaped in the past 30 days. Multiple sources could be selected.

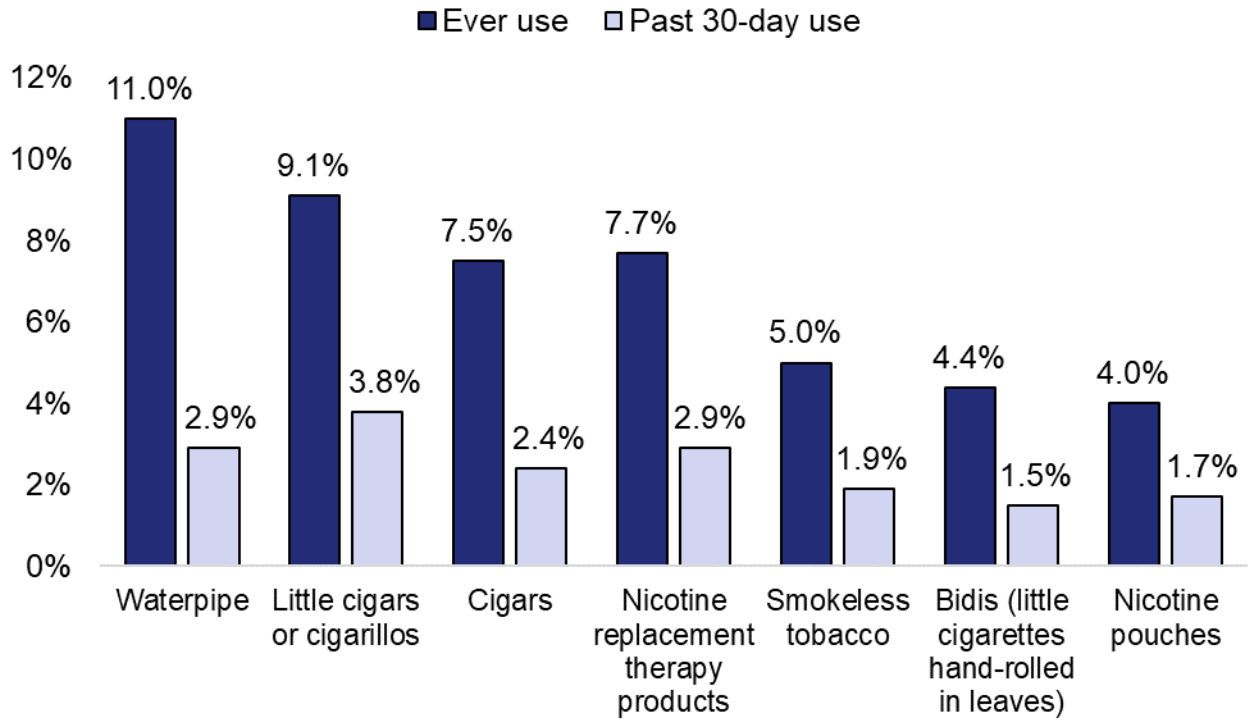
Among ITC Youth participants aged 16 to 19 years who had vaped in the past 30 days, the majority reported owning their own vaping product (70.8%). A substantial majority of under 18 year olds reported owning a vaping product (16 to 17 year olds: 64.3%). Among older young people, 78.8% of 18 year olds reported owning one, and 70.6% of 19 year olds reported owning one.

3.13 Other nicotine products

Awareness and use of heated tobacco products among young people is described in the chapter on heated tobacco products (chapter 14). Briefly, 2.2% of participants in the ITC Youth survey reported ever use, and 0.3% of participants in the ASH-Y survey reported current use.

The 2021 ITC Youth survey also asked about ever use and past 30-day use of different tobacco and nicotine products (Figure 23). Among young people aged 16 to 19 in England, 11.0% reported having ever used a waterpipe, 9.1% had ever used little cigars or cigarillos and 7.5% had ever used cigars. A very small proportion had ever used smokeless tobacco products (5.0%) or nicotine pouches (4.0%). There has been little change in past 30 day use of these products since 2017, apart from waterpipes, where past 30 day use was estimated at 5.8% in 2019, double the prevalence reported in 2021 (7). This may be due to the COVID-19 closure of bars, restaurants and festivals where waterpipes are typically used in England.

Figure 23. Ever use and past 30-day use of tobacco and nicotine products among young people aged 16 to 19, England, 2021 (ITC Youth, weighted data)



Notes: Unweighted base=4,298.

3.14 Conclusions

Data reported in this chapter were collected in February 2021 (from the ITC Youth survey), in March to April 2021 (from the 2021 ASH-Youth survey) and we also report top-line prevalence data from the ASH-Y 2022 survey carried out in February to March 2022. In response to the COVID-19 pandemic, schools were closed in England between the 4 January and 15 March 2021, and there were tight restrictions on social gatherings between the 4 January and 19 May 2021. Although no restrictions were in place during 2022 data collection, it is likely that there are ongoing effects of the 2 years of social restrictions on youth. So, conclusions in this chapter may be greatly affected by the impact of the COVID-19 regulations, resulting social restrictions and social disruption on youth.

2022 ASH-Y survey data (11 to 18 year olds) showed:

- current smoking prevalence (including occasional and regular) was 6.0% in 2022, compared with 4.1% in 2021 and 6.7% in 2020
- current vaping prevalence (including occasional and regular) was 8.6% in 2022, compared with 4.0% in 2021 and 4.8% in 2020

ITC Youth 2021 survey data (16 to 19 year olds) showed:

- current smoking prevalence (defined as smoking more than 100 cigarettes in their life and having smoked in the past 30 days) was 7.9% in 2021 (compared with 8.5% in February 2020 and 6.2% in August 2019)

- current vaping prevalence (defined as vaping on more than 10 days in their lifetime and having vaped in the past 30 days) was 9.1% in 2021 (compared with 9.4% in February 2020, and 7.7% in August 2019)

Overall, data from the 2021 ASH-Y and ITC Youth surveys were broadly similar for comparable age categories. Vaping among 19 year olds has been steadily increasing in the ITC Youth data over recent years.

The 2022 ASH-Y data suggest that overall nicotine use (via smoking and/or vaping) has increased over the past year, being 11.1% in 2022 compared with 6.2% in 2021; in 2015 the proportion was 7.7%.

Based on the socio-economic grade of 11 to 18 year olds in the 2022 ASH-Y survey the estimates for smoking and vaping prevalence were similar for the more advantaged groups in social grades A, B and C1 (5.8% for smoking, 8.4% for vaping) to more disadvantaged groups in social grades C2, D and E (5.4% for smoking, 8.1% for vaping). This was a departure from previous years. For example, in 2021, the estimates for smoking and vaping prevalence were higher among the more advantaged groups in social grades A, B and C1 (4.6% for smoking, 4.4% for vaping) than for the more disadvantaged groups in social grades C2, D and E (2.8% for smoking, 3.0% for vaping), similar to ASH-Y data from previous years.

The 2022 ASH-Y data indicated that most young people who had never smoked were also not currently vaping (98.3%). This was consistent with the 2021 ASH-Y and 2021 ITC-Youth data although the proportions were higher (99.2% and 99.1% respectively).

Disposable models were the most popular type of vaping device in the 2022 ASH-Y survey, used by 52.8% of 11 to 18 year olds who currently vaped, and 18.7% used tank models (which are reusable and rechargeable kits that users can refill with liquid). This was a stark difference from previous years where tank models were the most popular type of vaping device. For example, in 2021, only 7.8% of current vapers reported use of disposable models, whereas 41.0% used tank models.

Youth from the 2021 ITC survey reported an effect of COVID-19 on smoking and vaping behaviour: 8.0% of past year vapers reported quitting vaping and 15% reported cutting down due to the COVID-19 pandemic. However, 15% reported vaping more as an effect of the pandemic. Similar patterns were seen among those who had smoked in the past year, with 7% reporting quitting, 20% reporting cutting down, but 18% reporting smoking more.

These findings could contribute to the slight increase in former smokers (from 0.8 to 1.7%) and former vapers (from 4.6 to 8.6%) observed in the ITC Youth data between 2019 and 2021.

The main reasons for vaping were to “give it a try” (48.8%, 2021 ASH-Y), and “liking the flavours” (37.2%, ITC Youth). These reasons were most common among those who have never smoked or only tried smoking. Among youth who smoked, or had smoked, in the ITC youth survey, harm reduction, and quitting related reasons were common.

In the 2021 ASH-Y survey, most 11 to 18 year olds who had tried vaping had smoked first (38.7%), while 24.7% said they had vaped before they smoked and 29.7% said they had tried a vaping product and never tried smoking.

Fruit flavours were the most popular among current vapers (51.5% in 2021 ASH-Y). This was followed by “menthol/mint” (13.0%), then “chocolate/dessert/sweet/candy" flavours (9.3%), similar to data presented in our 2021 report.

Although it is illegal to sell vaping products to under 18 year olds, many under the age of 18 purchased and owned their own vaping devices. Among youth aged 11 to 17 from the 2021 ASH-Y survey, just under a quarter (24.8%) said that they were given products by friends, but substantial minorities also reported buying them, for example 22.1% said they bought them from newsagents, 22.1% online and 16.3% from a supermarket. Similarly, youth aged 16 to 17 who had vaped in the past 30 days from the ITC survey commonly reported being given products (37.5%). Many also reported buying products from shops (32.1%) or online (23.3%). Nearly two-thirds (64.3%) of 16 to 17 year olds from the ITC survey who had vaped in the past 30 days reported they owned a vaping product.

About a third (34.2%) of 11 to 18 year olds in the 2021 ASH-Y survey who currently vaped or had vaped in the past reported always using vaping products that contained nicotine and 20.4% reported always using nicotine-free products. Just over two-thirds (68.9%) of 16 to 19 year olds who had vaped in the past 30 days and had ever used vaping products with nicotine, reported using nicotine in their current vaping product and 12.3% said their vaping product did not contain nicotine.

In 2021, the most common nicotine strength used by 16 to 19 year olds in the ITC Youth survey who had vaped in the past 30 days was reported to be under 20mg/mL (64.0%); 17.2% reportedly used a strength between 20mg/mL and 49mg/mL and 5.6% reportedly used 50mg/mL or over. Compared to 2019 (19.6%), fewer participants reported they did not know the strength of their vaping liquid (7.3%). About half (53.1%) of 16 to 19 year olds who vaped in the past 30 days reportedly used nicotine salts, similar levels to those seen in 2019 (56.6%); 40.4% did not use nicotine salts and 6.5% were unsure. This has changed compared to 2019, where 30.6% did not use salts and 12.8% were unsure. Overall, there was higher awareness of the inclusion of nicotine and type of nicotine and fewer don’t know responses in 2021 compared to 2019.

Under half (42.8%) of 16 to 19 year olds in the 2021 ITC Youth survey who currently vaped did not feel addicted to vaping, but half (52.5%) said they felt a little or very addicted. In comparison, 14.5% of 16 to 19 year olds who currently smoked did not feel addicted to smoking, and 83.0% reported they felt a little or very addicted.

Just under a half (44.5%) of 16 to 19 year olds in the 2021 ITC Youth survey who currently vaped reported experiencing urges to vape almost daily or more than daily, with 16.8% reporting never experiencing an urge to vape. In comparison, 66.6% of youth who currently smoked reported urges to smoke daily or multiple times a day, with 4.7% reported never having urges to smoke.

Four in ten 11 to 18 year olds in the 2021 ASH-Y survey who currently vaped said they did not feel any urges to vape at all (41.5%), with 23.5% reporting strong or extremely strong urges to vape. In comparison, 24.3% of those who currently smoked reported no urge to smoke with 31.4% reporting strong, very strong or extremely strong urge to smoke.

Just over one-tenth (11.0%) of 16 to 19 year olds in the ITC Youth survey reported ever use of a waterpipe, 4.0% reported ever using nicotine pouches, and 5.0% reported ever using smokeless tobacco.

3.15 Implications

Vaping and smoking among youth appear to have decreased between 2020 and 2021 but then increased in 2022, hence it is important that trends continue to be monitored. The differences in estimates between the ASH-Y and ITC Youth surveys in 2021 are likely due to differences in the age demographics and a higher prevalence of vaping among 19 year olds who are included in the ITC Youth but not the ASH-Y. There are also possible lasting effects of the COVID-19 pandemic.

In 2022, higher vaping prevalence was reported across all age categories, therefore as mentioned in our previous reports, enforcement of age of sale regulations for vaping (and smoking) needs to be improved to reduce youth access to vaping products and cigarettes.

The dramatic increase in use of disposable products should be monitored with improved regulatory oversight. Also, the advertising, packaging and marketing of disposable products to young people should be investigated and, where appropriate, proportionate action taken to reduce appeal to young people.

A small majority of 16 to 19 year olds reported changing vaping and smoking behaviours in 2021 due to COVID-19 and these trends need to be closely monitored given the effects of the pandemic is ongoing.

Trends in reasons for use, types of vaping product used, and nicotine strength assessed in 2021 have remained broadly similar compared to trends prior to COVID-19.

Dependence on vaping as assessed in 2021 appears lower than on smoking for youth. Further research on dependence is needed including by type of vaping product used, nicotine type and nicotine strength.

3.16 References

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4 Vaping among adults

4.1 Objective

This chapter summarises the latest available survey data on vaping among adults in England. The focus is on vaping, with equivalent data on smoking reported where a comparison between vaping and smoking is appropriate and illustrative. This chapter reports vaping prevalence overall and by smoking status, as well as reasons for vaping, details on vaping behaviour, and urges to vape and smoke. Where data are available and group sizes allow, we present the data broken down by age, gender, region, social grade, ethnicity and vaping and smoking status. We also briefly report prevalence of selected other tobacco and nicotine products with heated tobacco products discussed in a separate chapter (chapter 14).

4.2 Surveys

The chapter uses survey data from the Smoking Toolkit Study (STS), Action on Smoking and Health – Adults (ASH-A), Opinions and Lifestyle Survey (OPN) and Annual Population Survey (APS) as described in chapter 2. Where available, the STS data are used (age 18+; sample size for January to September 2021 = 14,758) as they have the lowest risk of bias due to the size and representative nature of the sampling strategy (1). The ASH-A survey data (age 18+; sample size for England 2021 = 10,211) are presented alongside the STS data. The APS data are only used to report on smoking prevalence in England as APS does not provide information on vaping.

In May 2022 as we were finalising our report, we became aware that ASH was to publish a report from their 2022 ASH-A Survey findings around the same time as we would be publishing our report. For consistency across our 2 reports, we therefore incorporated top-line smoking and vaping prevalence data from the 2022 ASH-A survey (age 18+, sample size for England = 10,883, data collected February-March 2022) into this chapter. As the 2022 ASH-A survey data also identified a change in the types of vaping products used, and in line with issues identified by trading standards officers about disposables discussed in our Introduction chapter, we also included the 2022 ASH-A data on types of vaping products used. Given time constraints, we were unable to include other 2022 ASH-A data but the full report from the 2022 ASH-A survey will be available on the ASH website. While the STS data beyond September 2021 were also available, we were unable to include these updated data, but interested readers can find these on the STS website.

In this chapter, where appropriate, we compared data from different surveys conducted in 2021. Estimates sometimes differ between surveys. Surveys had different dates and modes of data collection, sample sizes, weighting methods and definitions (for example,

smoking status) that may explain differences. Also, some sample sizes (for example, adults from black and minority ethnic groups) were small and more sensitive to random variation.

Comparisons with previous years' data will be presented where illustrative. For the STS, yearly changes are presented with data from 2010 to 2021. Many variables of interest have been added to the STS more recently than 2010; where this is the case, the available data are presented. The ASH-A survey has run every year since 2012, and its data from 2012 to 2021 (and sometimes 2022) are used to report change over time.

Data collection for surveys was affected by the coronavirus (COVID-19) pandemic. In particular, the STS stopped collecting data from people aged 16 and 17 after February 2020, collected no data in March 2020, and changed the data collection modality from face-to-face to telephone interviews from April 2020 as described in chapter 2. To be consistent with our last report (2), we will only use STS data from people aged 18 and over when comparing data from 2010 to 2021. We will also note in the report which observed changes in the STS data trends might be related with a change in the modality of data collection after March 2020, although the representativeness of survey samples has not been affected by this change (3). The data collection modality has also changed for the APS—since April 2020, data for the survey has been collected by telephone only compared with earlier data collection using face to face and telephone interviews (4). Therefore, the APS smoking prevalence data for 2020 have been split by data collection modes.

It is also important to reiterate that the COVID-19 pandemic and associated restrictions had significant effect on health-related behaviours both in 2020 and 2021. In terms of smoking, a few noticeable changes in the STS data after the March 2020 lockdown were an increase in stop smoking attempts among smokers aged 18 to 34 and in successful cessation among past-year smokers of all ages (3); these changes did not differ by smokers' gender or social grade (5). Also, there was some evidence of an increase in use of remote smoking cessation support, while use of evidence-based support did not change (3). However, there was also some evidence of an increase in smoking prevalence among 18 to 34 year olds during April to July 2020 (a period of lockdown due to COVID-19) whereas prevalence was relatively stable among the older age groups (5). Jackson and others (5) offered various hypotheses for the increase in younger age groups including stress of the pandemic affecting different groups and COVID-19 health concerns being different across age groups. However, Jackson and others (5) also noted that the increased prevalence and increased quit attempts in the 18 to 34 year olds seemed discordant and warranted further research, although they suggested one explanation could be potential changes in demography if younger age groups were more likely to leave England during the pandemic and if smoking prevalence was lower among those that left (6, 7). Regarding vaping behaviour, an analysis of STS data between April and May 2020 reported that around 1 in 10 current vapers (19 out of 170) had tried to quit vaping

because of the COVID-19 outbreak (8). Nevertheless, for most vapers the main reason for wanting to quit vaping was not associated with COVID-19 (8, 9). An analysis of the Health Behaviours during the COVID-19 pandemic (HEBECO) online data found that among current vapers in the UK (n = 397), 9.7% (95% CI 6.8%-12.6%) reported vaping less than usual since COVID-19, 42.0% (37.2% to 46.9%) reported vaping more, and 48.3% (43.4% to 53.2%) reported no change. In this study, the increased vaping was more common among younger rather than older participants and among vapers who were suspected or diagnosed with COVID-19, although the latter association was based on the small number of confirmed COVID-19 cases and has not been examined prospectively (9).

The COVID-19 pandemic continues to affect smoking, vaping and many other health related behaviours in England. Therefore, its impact should be considered when interpreting recent changes in smoking or vaping trends.

4.3 Smoking and vaping prevalence among adults in England

Smoking prevalence

According to APS data, smoking prevalence in the first quarter of 2020 (13.5%; age 18+; sample size = 31,265) did not differ statistically significantly from the 2019 estimate of 13.9%. The latest APS smoking prevalence estimate for April to December 2020 was lower (12.1%; sample size = 88,897), but is not comparable to the first quarter of 2020 data due to the change in data collection modality (4). The OPN, which is the second largest survey available (age 16+; sample size = 71,286), showed that smoking prevalence in England was 14.5% in 2020; this was a decrease from 15.8% in 2019, but was not statistically significant (4).

For 2021, survey estimates for smoking prevalence among adults in England ranged from 12.7% in ASH-A to 14.9% in STS (table 1). Using the latest population data from the ONS (10) and lower and higher smoking prevalence rates from the ASH A and STS surveys, we can estimate that there were between 5.6 and 6.6 million smokers aged 18 and over in England in 2021.

Smoking prevalence in the ASH-A survey has fluctuated around 13% since 2020—it decreased by 1.1 percentage points in 2021 (from 13.8% in 2020 to 12.7%) and increased to 13.2% in March 2022. In the STS survey smoking prevalence remained similar between 2020 and 2021—14.8% in 2020 and 14.9% in the period from January to September 2021. The STS estimate of smoking prevalence for 2020 differs from the estimate in our previous report (2) because here we use a full year’s data for the 2020 smoking prevalence estimate.

Smoking prevalence trends over the past 12 years from 3 national surveys (STS, ASH-A and OPN) are shown in Figures 1a, 1b and 1c.

Table 1. Current smoking, ever tried and current vaping (%) among adults in 4 national surveys, England 2020, 2021 and 2022 (APS, OPN, STS and ASH-A; weighted data)

Smoking and vaping status	APS 2020 Q1* Age 18+	APS 2020 Q2-Q4* Age 18+	OPN 2020 Age 16+	STS 2021 Age 18+	ASH-A 2021 Age 18+	ASH-A 2022 Age 18+
Current smoking	13.5	12.1	14.4	14.9	12.7	13.2
Ever tried vaping	-	-	7.7	-	21.3	22.2
Current vaping	-	-	6.2	6.9	7.1	8.3
Unweighted bases	31,265	88,897	Smoking: 71,286 Vaping: 18,137	Smoking: 14,658 Vaping: 14,758	10,211	10,883

Notes: APS: Current smoking included people who had tried cigarettes and that said they still smoked ‘nowadays’.

OPN: Current smoking included people who had tried cigarettes and that said they still smoked ‘nowadays’. Current vaping included people who defined themselves as either daily users or occasional users of a vaping product. Ever tried vaping include people who reported have previously regularly vaped, those who vaped occasionally and those who tried vaping but did not go on to use the products.

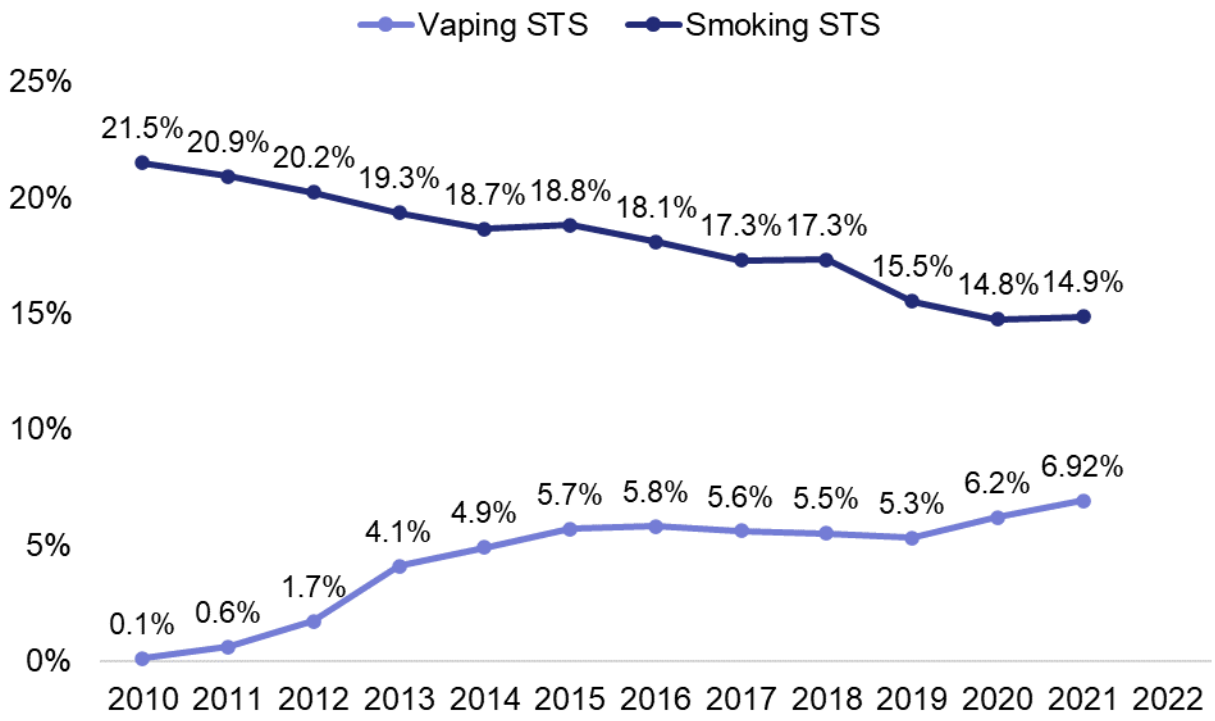
STS: Current smoking included people who said that they smoked daily or that they smoked, but less than daily. Current vaping included people who ‘currently vaped for any reason’. STS data available from January to September 2021. The unweighted bases for vaping and smoking differ because of missing data among small numbers of participants.

ASH-A: Current smoking included people who smoked daily as well as those who smoked, but not daily. Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped. Ever tried included people who had tried vaping and those who continued to vape.

* For the first quarter of 2020 (January to March), data for the APS survey were collected using face to face and telephone interviews. For the last 3 quarters in 2020 (April to December), data collection mode for the APS changed to telephone only due to the COVID-19 pandemic. Smoking prevalence rate after the change in data collection mode should be treated with caution (4).

Figures 1a, 1b and 1c. Current smoking and vaping prevalence among adults in 3 national surveys, England 2010 to 2022 (weighted data)

Figure 1a. Smoking Toolkit Study (STS)

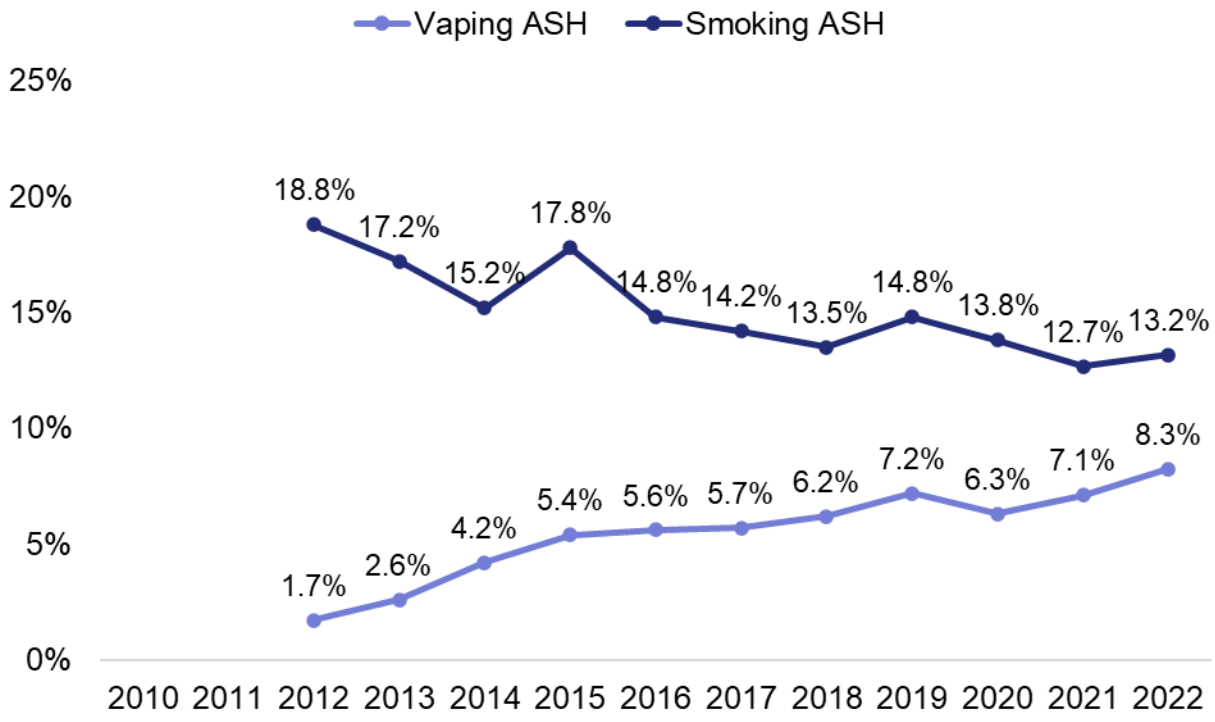


Notes: STS (18+): Unweighted bases smoking: 2010=24,268; 2011=21,299; 2012=20,832; 2013=21,658; 2014=19,733; 2015=19,642; 2016=20,063; 2017=20,036; 2018=20,402; 2019=20,380; 2020=18.378; 2021=14,658. Unweighted bases vaping: 2010=24,294; 2011=21,315; 2012=13,897; 2013=18,311; 2014=19,798; 2015=19,650; 2016=20,066; 2017=20,051; 2018=20,421; 2019=20,385; 2020=15,811; 2021 (January to September)=14,758.

Current smokers included people who said that they smoked daily or that they smoked, but less than daily.

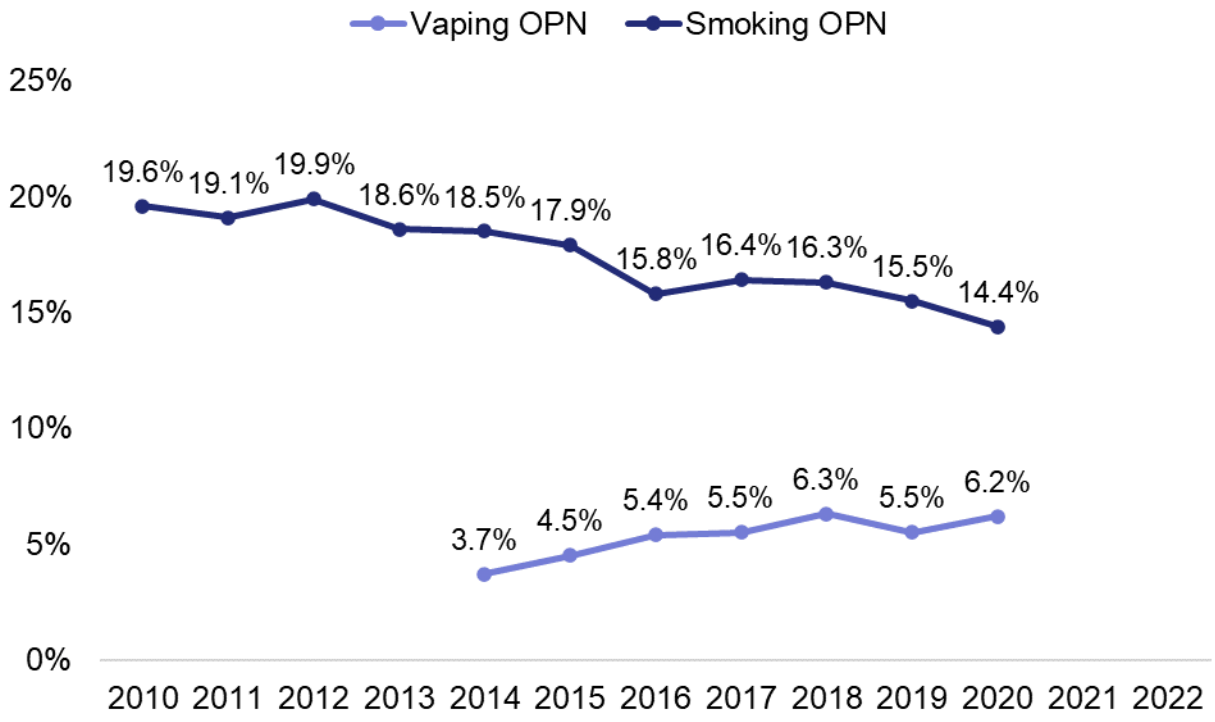
Current vapers included people who ‘currently vaped for any reason’. STS data available from January to September 2021, all previous years use the full year’s data.

Figure 1b. Action on Smoking and Health – Adults (ASH-A)



Notes: ASH-A (18+): Unweighted bases for both smoking and vaping: 2012=10,742; 2013=10,022; 2014=10,112; 2015=10,017; 2016=10,058; 2017=10,488; 2018=10,578; 2019=10,208; 2020=9,329; 2021=10,211; 2022=10,883. Current smoking included people who smoked daily as well as those who smoked, but not daily. Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped.

Figure 1c. Opinions and Lifestyle Survey (OPN)



Notes: OPN (16+): Unweighted base Smoking: 2014=9,320; 2015=8,139; 2016=7,713; 2017=7,122; 2018=9,620; 2019=6,511; 2020=71,286. Vaping: 2014=4,285; 2015=6,940; 2016=6,679 2017=6,079; 2018=6,619; 2019=6,509; 2020=18,137.

Current smoking included people who had tried cigarettes and that said they still smoked ‘nowadays’. Current vaping included people who defined themselves as either daily users or occasional users of a vaping product.

In the STS data, more than a fifth of 25 to 34 year olds (23.0%) and 18 to 24 year olds (21.6%) were current smokers compared with, for example, 8.2% of people aged over 65 (table 3a). In the 2021 ASH-A survey, current smoking was lowest among people from the oldest (55+) and the youngest (18 to 24) age groups—9.6% and 12.6%, respectively—compared with participants aged from 25 to 54. It is unclear why the smoking estimates for young adults' groups (18 to 24 and 25 to 34) differ markedly between the STS and ASH-A 2021 surveys, but the same difference was noted in our previous report, and differences in the composition of the unweighted samples between the 2 surveys remain the most likely reason for this discrepancy (2). The most notable differences between the 2022 and 2021 ASH-A surveys in smoking prevalence were: 14.6% among 18 to 24 year olds in 2022 compared with 12.6% in 2021; 15.6% among 45 to 54 year olds in 2022 compared with 13.5% in 2021; and 16.1% in 2022 among 35 to 44 year olds compared with 17.2% in 2021 (table 4).

In 2021, estimates of smoking prevalence were statistically significantly higher for men than for women with 16.0% (STS) and 14.2% (ASH-A) of men currently smoking compared with 13.7% and 11.3% of women respectively. This was similar in the 2022 ASH-A data (14.5% men, 11.9% women) (table 4).

The surveys in 2021 and 2022 did not show differences in smoking prevalence across regions. Social grade in both STS and ASH-A surveys used the classifications from the National Readership Survey (11) (table 2). The STS reported smoking prevalence to be 11.0% for people from A, B and C1 groups (ABC1) compared with 19.5% for people from C2, D and E groups (C2DE, $\chi^2(1)=159.5$, $p<0.001$), with a similar and statistically significant gradient in the 2021 ASH-A data (10.5% in ABC1 and 15.2% in C2DE groups, $\chi^2(1)=58.2$, $p<0.001$; table 3a and table 3b) and in the 2022 ASH-A data (10.2% ABC1 and 16.7% C2DE , $\chi^2(1)=94.6$, $p<0.001$; table 4).

In the STS, estimates of smoking prevalence were higher for people from white (15.1%) than from black and minority ethnic (13.2%, $\chi^2(1)=4.0$, $p=0.047$) groups. ASH-A data showed the opposite results—smoking prevalence was higher among people from black and minority ethnic groups (16.1%) than among people from white ethnic groups (12.0%, $\chi^2(1)=12.8$, $p<0.001$) in 2021 and 15.7% in black and minority ethnic and 12.7% in white ethnic groups in 2022 ($\chi^2(1)=11.4$, $p<0.001$) (table 4). Differences in the 2021 survey findings are likely due to different sampling and weighting methods and smaller sample sizes for the black and minority ethnic groups surveyed (table 3a and table 3b).

Table 2. Social grade classifications derived from the National Readership Survey (11)

Social grade	Description
A	Higher managerial, administrative or professional
B	Intermediate managerial, administrative or professional
C1	Supervisory, clerical and junior managerial, administrative or professional
C2	Skilled manual workers
D	Semi and unskilled manual workers
E	State pensioners, casual or lowest grade workers, unemployed with state benefits only

Table 3a. Smoking and vaping prevalence among adults by age, gender, region, social grade and ethnicity, England 2021 (STS, weighted data, unweighted counts)

	Current smoker % (n)	Current vaper % (n)
Total	14.9 (2,016)	6.9 (942)
Age		
18 to 24	21.6 (301)	10.7 (157)
25 to 34	23.0 (455)	11.1 (223)
35 to 44	16.8 (338)	7.6 (149)
45 to 54	12.9 (335)	6.6 (169)
55 to 64	11.5 (283)	5.9 (145)
65+	8.2 (304)	2.6 (99)
Statistical testing	$\chi^2(5)=326.3$, $p<0.001$	$\chi^2(5)=213.5$, $p<0.001$
Gender		
Male	16.0 (1,026)	7.8 (505)
Female	13.7 (990)	6.0 (437)
Statistical testing	$\chi^2(1)=6.6$, $p=0.010$	$\chi^2(1)=11.7$, $p<0.001$
Region		
North	15.1 (569)	8.3 (313)
Midlands	14.6 (375)	6.6 (165)
South	14.8 (1,072)	6.3 (464)
Statistical testing	$\chi^2(2)=0.5$, $p=0.760$	$\chi^2(2)=16.0$, $p<0.001$
Social grade		
ABC1	11.0 (945)	5.4 (468)
C2DE	19.5 (947)	8.8 (420)
Statistical testing	$\chi^2(1)=159.5$, $p<0.001$	$\chi^2(1)=42.3$, $p<0.001$
Ethnicity		
White	15.1 (1,794)	7.0 (837)
Black and minority ethnic groups	13.2 (208)	6.2 (97)
Statistical testing	$\chi^2(1)=4.0$, $p=0.047$	$\chi^2(1)=1.7$, $p=0.193$

Notes: STS (18+): Unweighted bases for smoking by age, gender and region = 14,658; social grade = 13,764; ethnicity = 14,551. Unweighted bases for vaping by age, gender

and region = 14,758; social grade = 13,848; ethnicity = 14,644. Eighty-six people defined their gender in another way and 114 refused to report or did not know their ethnic origin. Current smoker included people who said that they smoked daily or that they smoked, but less than daily. Current vaper included people who 'currently vaped for any reason'. STS data available from January to September 2021.

Table 3b. Smoking and vaping prevalence among adults by age, gender, region, social grade and ethnicity, England 2021 (ASH-A, weighted data, unweighted counts)

	Current smoker % (n)	Current vaper % (n)
Total	12.7 (1,228)	7.1 (690)
Age		
18 to 24	12.6 (127)	5.4 (55)
25 to 34	14.6 (204)	8.2 (113)
35 to 44	17.2 (273)	10.0 (156)
45 to 54	13.5 (209)	8.6 (134)
55 to 64	9.6 (415)	5.3 (232)
Statistical testing	$\chi^2(4)=59.7$, $p<0.001$	$\chi^2(4)=41.6$, $p<0.001$
Gender		
Male	14.2 (632)	8.1 (362)
Female	11.3 (596)	6.2 (328)
Statistical testing	$\chi^2(1)=12.3$, $p<0.001$	$\chi^2(1)=9.6$, $p=0.002$
Region		
North	12.4 (343)	7.8 (209)
Midlands	12.1 (230)	7.7 (147)
South	13.0 (655)	6.6 (334)
Statistical testing	$\chi^2(2)=0.5$, $p=0.77$	$\chi^2(2)=4.9$, $p=0.086$
Social grade		
ABC1	10.5 (609)	6.4 (367)
C2DE	15.2 (619)	7.9 (323)
Statistical testing	$\chi^2(1)=58.2$, $p<0.001$	$\chi^2(1)=12.7$, $p<0.001$
Ethnicity		
White	12.0 (1,010)	6.9 (578)
Black and minority ethnic groups	16.1 (169)	8.5 (91)
Statistical testing	$\chi^2(1)=12.8$, $p<0.001$	$\chi^2(1)=4.0$, $p=0.045$

Notes: ASH-A (18+): Unweighted base for age, gender, region and social grade = 10,211; ethnicity = 9,855. Five participants selected 'preferred not to say' when asked about their ethnicity. Current smoker included people who smoked daily as well as those who smoked, but not daily. Current vaper included people who had tried vaping and who still vaped, excluding those who no longer vaped. In statistical testing, degrees of freedom might differ due to "Don't know" or 'Prefer not to say' responses.

Table 4. Smoking and vaping prevalence among adults by age, gender, region, social grade and ethnicity, England 2022 (ASH-A, weighted data, unweighted counts)

	Current smoker % (n)	Current vaper % (n)
Total	13.2% (1,415)	8.3 (901)
Age		
18 to 24	14.6 (262)	11.0 (194)
25 to 34	14.6 (227)	10.2 (159)
35 to 44	16.1 (286)	10.5 (189)
45 to 54	15.6 (239)	10.4 (160)
55+	10.0 (401)	4.9 (199)
Statistical testing	$\chi^2(4)=66.2$, $p<0.001$	$\chi^2(4)=107.1$, $p<0.001$
Gender		
Male	14.5 (741)	9.1 (467)
Female	11.9 (674)	7.5 (434)
Statistical testing	$\chi^2(1)=14.7$, $p<0.001$	$\chi^2(1)=7.0$, $p=0.008$
Region		
North	12.9 (392)	9.7 (289)
Midlands	13.9 (294)	9.5 (208)
South	13.1 (729)	7.0 (404)
Statistical testing	$\chi^2(2)=0.7$, $p=0.713$	$\chi^2(2)=21.8$, $p<0.001$
Social grade		
ABC1	10.2 (647)	7.3 (465)
C2DE	16.7 (768)	9.4 (436)
Statistical testing	$\chi^2(1)=94.6$, $p<0.001$	$\chi^2(1)=14.6$, $p<0.001$
Ethnicity		
White	12.7 (1,177)	8.2 (760)
Black and minority ethnic groups	15.7 (238)	8.8 (139)
Statistical testing	$\chi^2(1)=11.4$, $p<0.001$	$\chi^2(1)=1.9$, $p=0.165$

Notes: ASH-A (18+): Unweighted base for age, gender, region and social grade = 10,883; ethnicity = 10,871. Twelve participants selected ‘preferred not to say’ when asked about their ethnicity. Current smoker included people who smoked daily as well as those who smoked, but not daily. Current vaper included people who had tried vaping and who still vaped, excluding those who no longer vaped.

Vaping prevalence

Estimates of current vaping prevalence among adults aged 18+ in England ranged from 6.9% (STS data) to 7.1% (ASH-A) in 2021 (Figures 1a, 1b and 1c, table 1). Using the 2 survey estimates of current vaping prevalence and the most recent population data (10) we can estimate that there were 3.1 to 3.2 million adult vapers in England in 2021 (Figures 1a, 1b and 1c, table 1).

Vaping prevalence increased between 2010 and 2015, fluctuated until 2019 and then increased again (Figures 1a, 1b and 1c). The uptick in vaping prevalence in the STS survey has been noted since 2019 (5.3%), from which it increased to 6.2% in 2020 and again to 6.9% in 2021. In the ASH-A survey, change in vaping prevalence has been similar to the STS data—vaping among adults in England increased from 6.3% in 2020 to 7.1% in 2021, and to 8.3% in 2022.The continuing COVID-19 pandemic and further lockdowns may have contributed to an increase in vaping since 2020, but this requires further research.

In 2021, the STS reports 10.7% and ASH-A 5.4% vaping prevalence among 18 to 24 year olds, which is likely to reflect the discrepancies between the surveys in smoking prevalence for this age group and differences in the compositions of the samples (table 3a and table 3b). In the 2022 ASH-A survey, vaping prevalence was higher across all age groups than in 2021, with the biggest difference among 18 to 24 year olds (5.4% in 2021 and 11.0% in 2022) (table 4).

In 2021, vaping prevalence among men (7.8% for STS and 8.1% for ASH-A) was statistically significantly higher than among women (6.0% and 6.2% respectively) and this difference remained in the 2022 ASH-A data (9.1% vs 7.5%). In 2021, both STS and ASH-A surveys estimated vaping prevalence to be highest in the north of England (8.3% and 7.8% respectively) compared with the Midlands (6.6% and 7.7%) and the south (6.3% and 6.6%)—the difference, however, was statistically significant only for STS groups ($\chi^2(2)=16.0$, $p<0.001$; table 3a and table 3b); in the 2022 ASH-A data, the regional differences were statistically significant (9.7% north, 9.5% Midlands, 7.0% south; table 4).

Similar to smoking, vaping prevalence was statistically significantly higher among C2DE social grades (8.8% for STS and 7.9% for 2021 ASH-A) compared with ABC1 (5.4% and 6.4% for STS and 2021 ASH-A respectively); this statistically significant difference was also evident in the 2022 ASH-A data (9.4% among C2DE and 7.3% among ABC1). In terms of ethnicity, vaping prevalence in the STS was 7.0% for people from white ethnic groups and 6.2% for black and minority ethnic groups (non-significant statistical difference), whereas in the 2021 ASH-A survey data, it was 6.9% for white ethnic groups and statistically significantly higher at 8.5% for black and minority ethnic groups; in the 2022 ASH-A survey, vaping prevalence was 8.2% for white ethnic groups and 8.8% for black and minority ethnic groups. Differences in the 2021 surveys are likely due to different sampling and weighting methods and smaller sample sizes for the black and minority ethnic groups surveyed.

Smoking prevalence was higher than vaping prevalence across all age, gender, region social grade and ethnicity groups (table 3a and table 3b; table 4).

4.4 Vaping by smoking status

In 2021, 17.4% (ASH-A) and 22.0% (STS, January to September 2021) of current smokers vaped (table 5); the ASH-A 2022 data indicated this figure to be 20.9%. The STS estimate in 2021 was nearly 2 percentage points higher than the previous year's estimates (22.0% and 20.1% in January to October 2020, respectively). Vaping prevalence among former smokers was higher than in the previous year—13.4% in 2021 and 14.3% in 2022 for ASH-A (10.9% in 2020) and 11.6% in 2021 for STS (11.0% in January to October 2020). Current vaping prevalence among never smokers remains low and was under 1% across surveys in 2021, and, according to the 2022 ASH-A survey, 1.3% in 2022.

Table 5. Current vaping prevalence by smoking status among adults (age 18+) in 2 national surveys, England 2021 and 2022 (weighted data, unweighted counts)

Smoking status	STS 2021 % (n)	ASH-A 2021 % (n)	ASH-A 2022 % (n)
Never smokers	0.6 (50)	0.7 (31)	1.3 (89)
Former smokers	11.6 (412)	13.4 (446)	14.3 (504)
Current smokers	22.0 (480)	17.4 (213)	20.9 (308)
Unweighted bases	14,658	10,211	10,883

Notes: STS (18+): Current vaping included people who 'currently vaped for any reason'. Never smokers included people who had never regularly smoked for a year or more. Former smokers included those who had stopped smoking completely but who had smoked for a year or more in the past. Current smokers included people who said that they smoked daily or that they smoked, but less than daily. STS data available from January to September 2021.

ASH-A (18+): Current vaping included people who had tried vaping who still vaped, excluding those who no longer vaped. Never smokers included people who responded to a question about smoking with 'I have never smoked'. Former smokers included those who said that they used to smoke, but who had 'given up now'. Current smokers included people who smoked daily as well as those who smoked, but not daily.

Statistically significant differences were identified in vaping prevalence between former and current smokers across different age groups in both adult surveys in 2021 (table 6a and table 6b). The numbers of never-smoking vapers were too small to draw any conclusions. The STS data identified highest vaping rates among the youngest former (31.5%) and current (26.7%) smokers, while the 2021 ASH-A survey found most vapers among 25 to 34 year old former smokers (21.9%) and among 35 to 44 year old current smokers (20.1%). In both surveys, lowest vaping rates were among the oldest former and current smokers. As in the last report (2), rates of vaping among current smokers in the STS data were similar for women and for men, but in the ASH-A data vaping among current smokers differed statistically significantly (19.9% men, 14.5% women). Vaping among former and current smokers was similar across region and social grades. For ethnicity, ASH-A data indicated statistically significantly higher vaping rates among former and current smokers from black and minority ethnic groups, but the numbers involved were too small to draw firm conclusions, and the same statistically significant difference was not identified in the STS data (table 6a and table 6b).

In 2022 ASH-A data, the prevalence of never (1.3%), former (14.3%) and current (20.9%) smokers who vape were higher than in 2021 when they were 0.7%, 13.4% and 17.4% respectively (table 7). Compared with 2021, the biggest differences in vaping prevalence were among the youngest 18 to 24 year old age group—by 11.2 percentage points among former smokers (to 26.9% in 2022) and by 14.6 percentage points among current smokers (to 34.4% in 2022). In contrast to 2021 data, no statistically significant differences were found in vaping prevalence by gender and smoking status in 2022 ASH-A survey—this was likely due to the higher vaping prevalence among female smokers in 2022 (19.1%) than in 2021 (14.5%). Other discernible changes in vaping prevalence since 2021 included: a 9.1 percentage point higher vaping prevalence among current smokers in north England; a 3.1 (within ABC1) and 4.0 (within C2DE) percentage point higher vaping prevalence among current smokers; and 6.7 percentage point lower vaping prevalence among former smokers from black and minority ethnic groups (table 6a and table 6b; table 7).

Table 6a. Current vaping prevalence by smoking status among adults by age, gender, region, social grade and ethnicity, England 2021 (STS weighted data, unweighted counts)

	Never regularly smoked* % (n)	Former smokers % (n)	Current smokers % (n)
Total	0.6 (50)	11.6 (412)	22.0 (480)
Age			
18 to 24	1.7 (18)	31.5 (47)	26.7 (92)
25 to 34	0.9 (12)	24.0 (85)	25.9 (126)
35 to 44	0.6 (7)	14.3 (63)	21.6 (79)
45 to 54	0.4 (7)	11.8 (85)	21.8 (77)
55 to 64	0.2 (2)	11.5 (82)	19.7 (61)
65+	0.3 (4)	3.5 (50)	12.0 (45)
Statistical testing	-	χ^2 (5) = 23.0, p<0.001	
Gender			
Male	0.8 (29)	12.7 (227)	21.9 (249)
Female	0.5 (21)	10.4 (185)	22.1 (231)
Statistical testing	-	χ^2 (1) = 0.9, p=0.336	
Region			
North	0.5 (12)	15.1 (150)	24.4 (151)
Midlands	0.7 (12)	13.0 (74)	19.1 (79)
South	0.6 (26)	9.3 (188)	21.8 (250)
Statistical testing	-	χ^2 (2) = 3.8, p=0.151	
Social grade			
ABC1	0.5 (26)	10.0 (217)	21.5 (225)
C2DE	0.8 (19)	13.3 (175)	22.6 (226)
Statistical testing	-	χ^2 (1) = 2.5, p=0.113	
Ethnicity			
White	0.7 (44)	11.2 (373)	21.7 (420)
Black and minority ethnic groups	0.4 (5)	15.8 (37)	23.1 (55)
Statistical testing	-	χ^2 (1) = 1.5, p=0.214	

Notes: STS (18+): Unweighted base for age, gender and region = 14,658; social grade = 13,764; ethnicity = 14,551. Eighty-six people defined their gender in another way and 114 refused to report or did not know their ethnic origin.

Current vaper included people who ‘currently vaped for any reason’. Never regularly smoked included people who had never smoked for longer than one year. Former smokers included those who had stopped smoking completely but who had smoked for a year or more in the past. Current smokers included people who said that they smoked daily or that they smoked, but less than daily. STS data available from January to September 2021.

* Columns with 50 or fewer participants were not included in statistical testing as they do not represent a wide enough cross-section of the target population to be considered statistically reliable.

Table 6b. Current vaping prevalence by smoking status among adults by age, gender, region, social grade and ethnicity, England 2021 (ASH-A weighted data, unweighted counts)

	Never smokers* % (n)	Former smokers % (n)	Current smokers % (n)
Total	0.7 (31)	13.4 (446)	17.4 (213)
Age			
18 to 24	-	15.7 (17)	19.8 (26)
25 to 34	-	21.9 (68)	17.5 (37)
35 to 44	-	20.2 (97)	20.1 (53)
45 to 54	-	17.2 (91)	18.1 (39)
55 to 64	-	8.5 (173)	14.2 (58)
Statistical testing	-	χ^2 (4) = 22.4, p<0.001	
Gender			
Male	-	13.6 (218)	19.9 (126)
Female	-	13.2 (228)	14.5 (87)
Statistical testing	-	χ^2 (1) = 6.1, p=0.014	
Region			
North	-	15.1 (142)	17.3 (59)
Midlands	-	12.8 (85)	24.2 (53)
South	-	12.7 (219)	15.2 (101)
Statistical testing	-	χ^2 (2) = 3.2, p=0.199	
Social grade			
ABC1	-	11.9 (224)	20.5 (121)
C2DE	-	15.0 (222)	15.0 (92)
Statistical testing	-	χ^2 (1) = 2.5, p=0.114	
Ethnicity			
White	-	12.9 (392)	15.9 (162)
Black and minority ethnic groups	-	20.1% (43)	25.1 (44)
Statistical testing	-	χ^2 (1) = 15.7, p<0.001	

Notes: ASH-A (18+): Unweighted base for age, gender, region, social grade = 10,211; ethnicity = 9,855.

Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped. Never smokers included people who responded to a question about smoking with ‘I have never smoked’. Former smokers included those who said that they used to smoke, but who had ‘given up now’. Current smokers included people who smoked daily as well as those who smoked, but not daily. Five participants selected ‘prefer not to say’ when asked about their ethnicity.

* Columns with fewer than 50 participants have not been broken down by socio-demographic characteristics and were not included in statistical testing as they do not represent a wide enough cross-section of the target population to be considered statistically reliable.

Table 7. Current vaping prevalence by smoking status among adults by age, gender, region, social grade and ethnicity, England 2022 (ASH-A weighted data, unweighted counts)

	Never smokers % (n)	Former smokers % (n)	Current smokers % (n)
Total	1.3 (89)	14.3 (504)	20.9 (308)
Age			
18 to 24	3.6 (47)	26.9 (58)	34.4 (89)
25 to 34	2.3 (23)	24.7 (83)	23.1 (53)
35 to 44	1.0 (10)	21.4 (112)	23.2 (67)
45 to 54	0.6 (4)*	19.5 (108)	20.1 (48)
55+	0.3 (5)	7.7 (143)	12.9 (51)
Statistical testing	χ^2 (8) = 117.7, p<0.001		
Gender			
Male	1.5 (44)	14.6 (250)	22.5 (173)
Female	1.2 (45)	14.0 (254)	19.1 (135)
Statistical testing	χ^2 (2) = 3.5, p=0.171		
Region			
North	1.5 (27)	16.5 (159)	26.4 (103)
Midlands	2.1 (28)	16.0 (105)	24.1 (75)
South	1.0 (34)	12.7 (240)	16.9 (130)
Statistical testing	χ^2 (4) = 6.6, p=0.162		
Social grade			
ABC1	1.3 (53)	12.9 (254)	23.6 (158)
C2DE	1.4 (36)	15.8 (250)	19.0 (150)
Statistical testing	χ^2 (2) = 2.6, p=0.279		
Ethnicity			
White	1.1 (64)	14.4 (458)	19.4 (238)
Black and minority ethnic groups	2.3 (24)	13.4 (45)	28.4 (70)
Statistical testing	χ^2 (2) = 38.2, p<0.001		

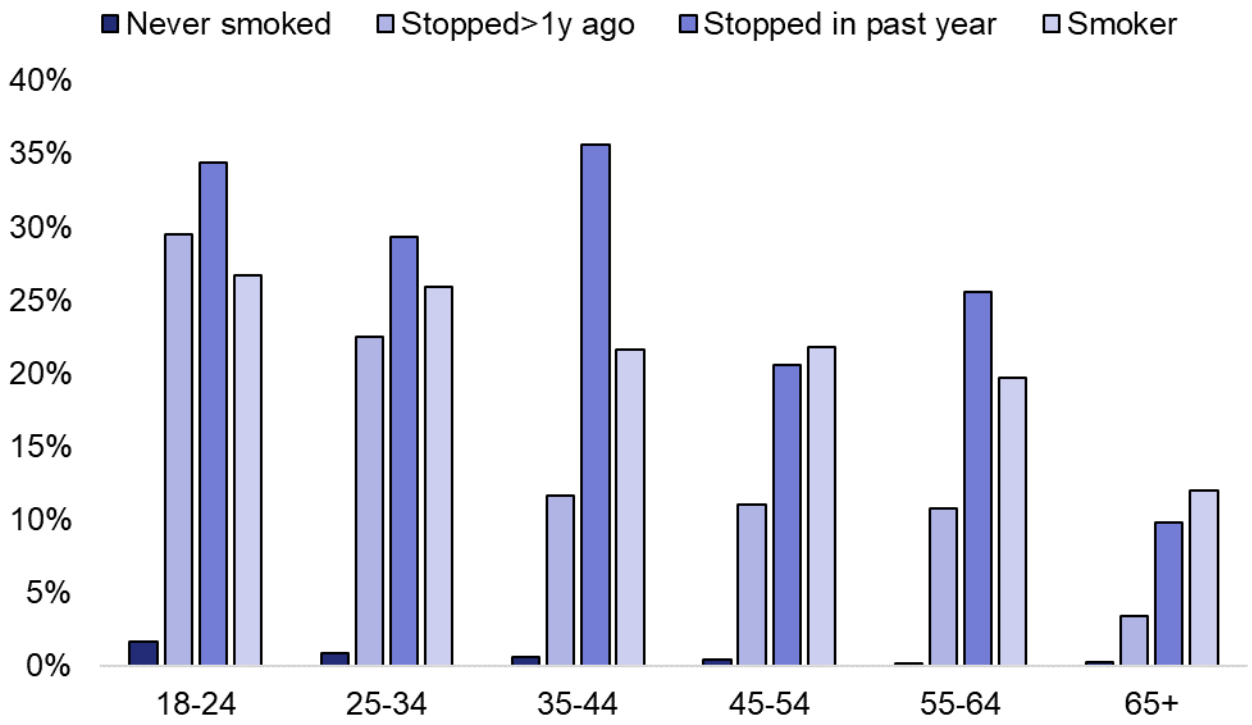
Notes: ASH-A (18+): Unweighted base for age, gender, region, social grade = 10,883; ethnicity = 10,871.

Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped. Never smokers included people who responded to a question about smoking with ‘I have never smoked’. Former smokers included those who said that they used to smoke, but who had ‘given up now’. Current smokers included people who smoked daily as well as those who smoked, but not daily. Twelve participants selected ‘prefer not to say’ when asked about their ethnicity.

* A cell with expected count less than 5 was included in χ^2 testing—the outcome might not be statistically reliable.

Former smokers in the STS survey were differentiated between long-term (had stopped smoking for longer than a year) and short-term former smokers (had stopped smoking in the past year); vaping prevalence was 9.9% among long-term former smokers and 27.9% among short-term former smokers. Figure 2 illustrates vaping prevalence among these groups. Among long-term former smokers, vaping prevalence was higher in those aged younger than 35 years. Among recent former smokers, vaping prevalence was spread across the age range but more common among those aged 18 to 44 years. Among people who had never smoked, vaping prevalence was low across all ages (1.7% among 18 to 24 year olds).

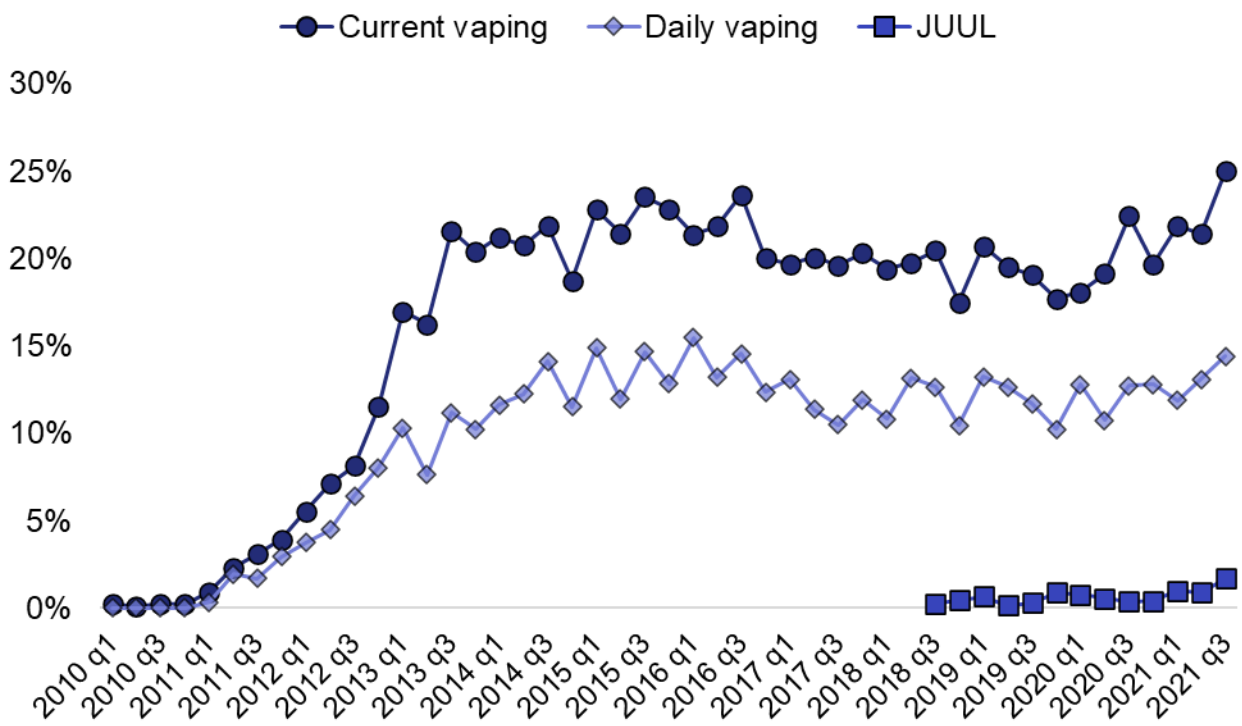
Figure 2. Current vaping prevalence by smoking status and by age, England 2021 (STS, weighted data)



Notes: Age 18+; Unweighted base = 14,658. Current vaping included people who ‘currently vaped for any reason’. Never smoked included people who had never smoked for longer than one year. Former smokers included those who had stopped smoking completely but who had smoked for a year or more in the past; and here was split between those who quit smoking over, and under one year ago. Current smokers included people who said that they smoked daily or that they smoked, but less than daily. STS data available from January to September 2021.

Figure 3 illustrates changes in current and daily vaping prevalence among past-year smokers (combining current smokers with short-term former smokers) between 2010 and 2021 (STS). It suggests a slight, but steady, decline in current vaping among past year smokers since 2015 to 2016, followed by an increase since the beginning of 2020. This uptick among current and daily vapers is likely due to an increase in past- year smokers vaping during the COVID-19 pandemic. In the third quarter of 2021, current vaping among past-year smokers has reached the highest point (25.0%) since STS data collection has started with 14.4% vaping daily. JUUL use by past-year smokers also increased to 1.7% in the third quarter of 2021.

Figure 3. Vaping prevalence and use of JUUL among smokers and recent (less than one year) former smokers, England 2011 to 2021 (STS, weighted data)



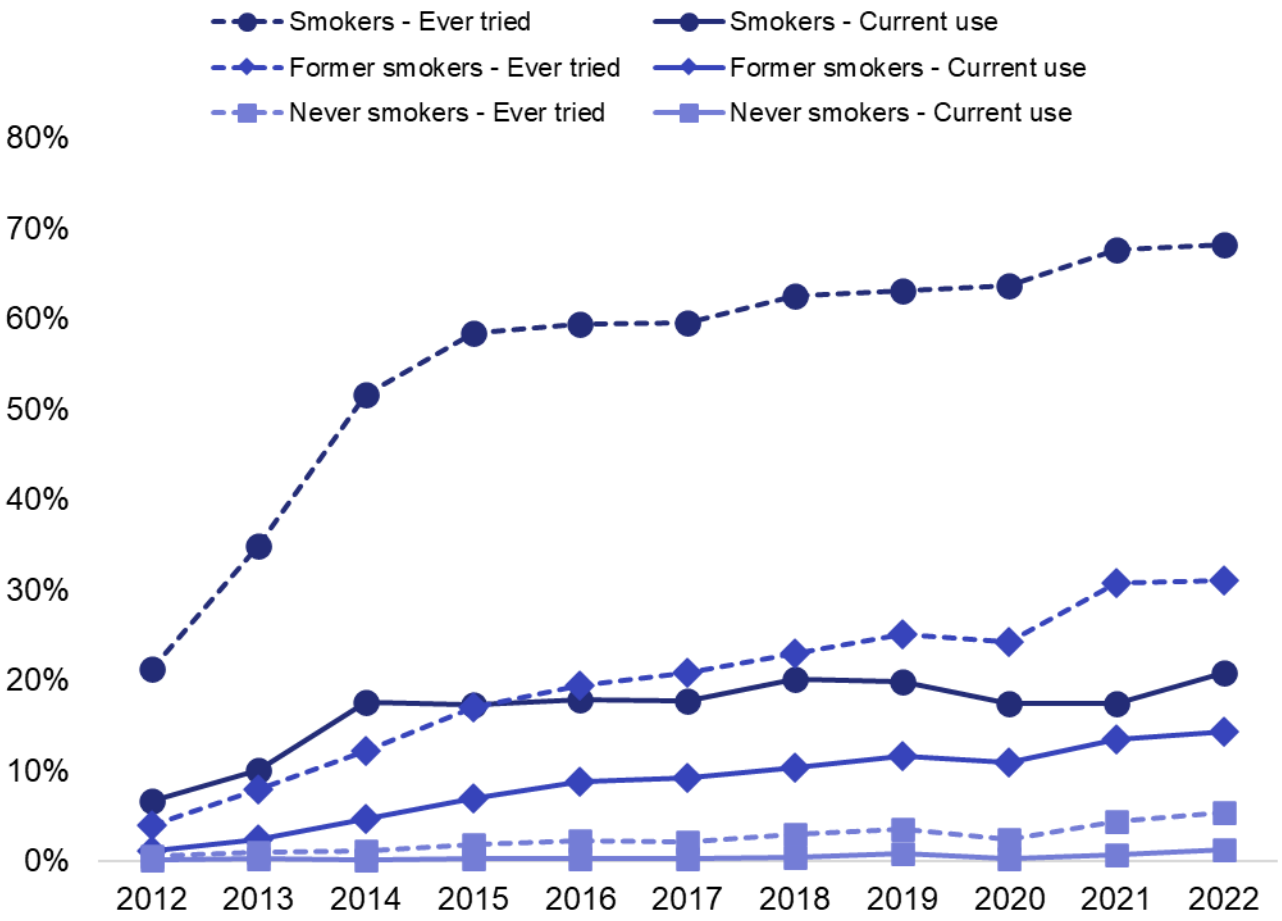
Notes: Age 18+; Unweighted bases vaping: 2010=6,005; 2011=5,110; 2012=3.204; 2013=3,556; 2014=3,711; 2015=3,621; 2016=3,407; 2017=3,225; 2018=3,311; 2019=2,994; 2020=3,332, 2021=3,605 (to September). Unweighted bases JUUL: 2010=6,005; 2011=5,191; 2012=5,063; 2013=4,760; 2014=4,203; 2015=4,147; 2016=3,922; 2017=3,651; 2018=3,755; 2019=3,408; 2020 =3,332, 2021=3,605 (January to September).

Recent former smokers included those who had stopped smoking completely, who had previously smoked for a year or more and who quit smoking under one year ago. Current smokers included people who said that they smoked daily or that they smoked, but less than daily. Current vaping included people who ‘currently vaped for any reason’. Daily vaping included people who reported currently using a vaping product and who reporting using nicotine every day. JUUL use included current use of JUUL for any reason. 2021

data available from January to September. The full year's data were used for all other years.

The proportion of smokers who currently use vaping products has remained relatively stable since 2014 (ASH-A, figure 4), dipping slightly in 2020, similar to vaping prevalence among former and never smokers. This decrease was likely due to concerns about the 'EVALI' outbreak in the US in late 2019 and early 2020 (12) (see also chapter 1). However, vaping among former smokers and trial of vaping products by never, former and current smokers seem to have slightly increased since 2020, which might have been associated with the COVID-19 pandemic and related national lockdowns. Small upticks are evident in current use among smokers, former smokers and never smokers in the ASH 2022 data (figure 4).

Figure 4. Ever tried and current use of vaping products among adults by smoking status, England 2012 to 2022 (ASH-A, weighted data)



Notes: Age 18+; Unweighted bases: 2012=10,742; 2013=10,022; 2014=10,112; 2015=10,017; 2016=10,058; 2017=10,488; 2018=10,578; 2019=10,208; 2020=9,329; 2021=10,211; 2022=10,883.

Never smoked included people who responded to a question about smoking with ‘I have never smoked’. Former smokers included those who said that they used to smoke, but who had “given up now”. Current smokers included people who smoked daily as well as those who smoked, but not daily. Ever tried vaping included people who had tried vaping and those who continued to vape. Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped.

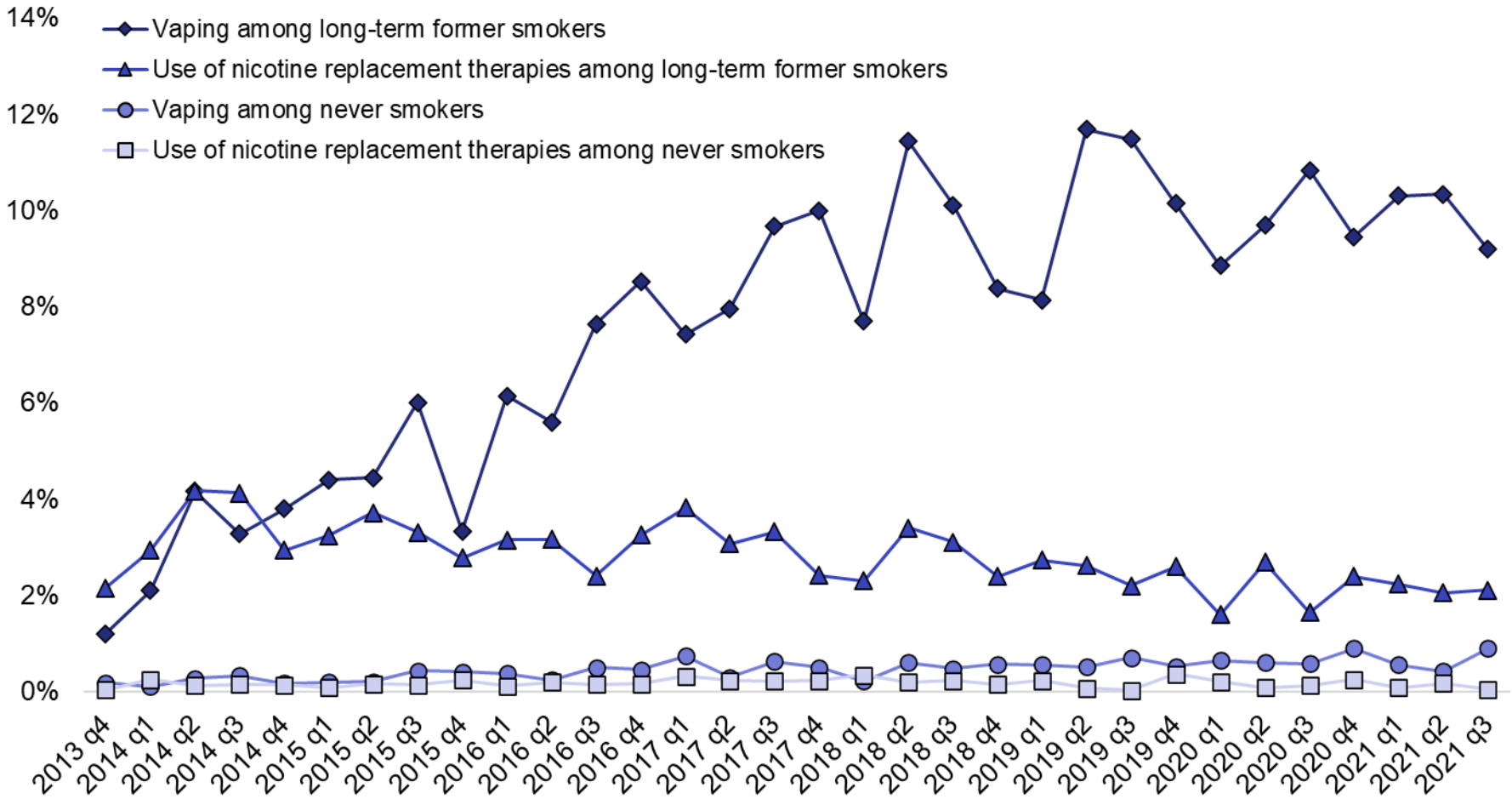
Vaping and NRT use by former and never smokers

Use of vaping and nicotine replacement therapy (NRT) products by long-term (more than one year) former smokers indicates whether those who quit smoking either continue or initiate using nicotine once they have successfully quit smoking. Trends of NRT and vaping product use by long term former smokers has remained similar since the last year with around 2.1% using NRT and 9.9% using vaping products in 2021 (figure 5). Numbers of NRT and vaping product users among never smokers in 2021 remained too small (0.1% and 0.6% respectively) for drawing further conclusions.

Use of NRT and vaping products by long term former smokers by age, gender, region, SES and ethnicity is reported in table 8.

Among long-term former smokers, vaping appeared to be more common in younger age groups (as noted in Section 4.4), in the North of England and the Midlands and among people from socio-economic grades C2DE. NRT use among long-term former smokers did not differ by the comparison groups, and numbers of never smokers who vape or use NRT were too small to draw any conclusions (table 8).

Figure 5. Use of vaping products and nicotine replacement therapy by never smokers and long-term (more than one year) former smokers, England 2014 to 2021 (STS, weighted data)



Notes: Age 18+; Unweighted bases: 2013=14,364; 2014=15,495; 2015=15,495; 2016=15,642; 2017=16,385; 2018=15,574; 2019=16,972; 2020=15,319; 2021 (to September)=12,109. Current vaper included people who ‘currently vaped for any reason’. Use of nicotine replacement therapy (NRT) included people who were currently using NRT. 2021 data available from January to September. The full year’s data were used for all other years.

Table 8: Vaping and NRT prevalence by never smokers and long-term (more than one year) former smokers by age, gender, region, social grade and ethnicity, England 2021 (STS, weighted data)

	Vaping		NRT use	
	Never smokers % (n)	Long-term former smokers % (n)	Never smokers* % (n)	Long-term former smokers % (n)
Total	0.6 (50)	9.9 (324)	0.1 (7)	2.1 (79)
Age				
18 to 24	1.7 (18)	29.5 (25)	-	3.1 (3)**
25 to 34	0.9 (12)	22.5 (61)	-	2.2 (7)
35 to 44	0.6 (7)	11.6 (46)	-	2.8 (12)
45 to 54	0.4 (7)	11.0 (73)	-	2.6 (18)
55 to 64	0.2 (2)	10.8 (72)	-	2.7 (19)
65+	0.3 (4)	3.4 (47)	-	1.2 (20)
Statistical testing	-	χ^2 (5) = 159.0, p<0.001	-	χ^2 (5) = 7.6, p=0.181
Gender				
Male	0.8 (29)	10.8 (179)	-	2.1 (39)
Female	0.5 (21)	9.0 (145)	-	2.1 (40)
Statistical testing	-	χ^2 (1) = 1.6, p=0.207	-	χ^2 (1) = 0.2, p=0.669
Region				
North	0.5 (12)	12.6 (116)	-	1.6 (17)
Midlands	0.7 (12)	11.1 (57)	-	2.7 (17)
South	0.6 (26)	8.1 (151)	-	2.2 (45)
Statistical testing	-	χ^2 (2) = 11.9, p=0.003	-	χ^2 (2) = 2.0, p=0.365
Social grade				
ABC1	0.5 (26)	8.5 (172)	-	2.4 (47)
C2DE	0.8 (19)	11.6 (139)	-	1.6 (23)
Statistical testing	-	χ^2 (1) = 4.3, p=0.039	-	χ^2 (1) = 1.2, p=0.278
Ethnicity				
White	0.7 (44)	9.7 (296)	-	2.1 (73)
Black and minority ethnic groups	0.4 (5)	13.3 (26)	-	1.3 (4)**
Statistical testing	-	χ^2 (1) = 2.2, p=0.139	-	χ^2 (1) = 0.1, p=0.721

Notes: Age 18+; Unweighted bases for never smokers: for age, gender and region = 8,471; social grade = 7,928; ethnicity = 8,409.

Unweighted bases for long-term former smokers: for age, gender and region = 3,609; social grade = 3,438; ethnicity = 3,581. Current vaper included people who ‘currently

vaped for any reason'. Use of NRT included people who were currently using NRT. Never smokers included people who had never smoked for longer than one year. Long-term former smokers included those who had stopped smoking completely over one year go, but who had smoked for a year or more in the past. STS data available from January to September 2021.

* Columns with 50 or fewer participants were not included in statistical testing as they do not represent a wide enough cross-section of the target population to be considered statistically reliable.

** A cell with expected count less than 5 was included in χ^2 testing—the outcome might not be statistically reliable.

Duration of use

Since 2018, the ASH-A survey has asked participants about the length of time they have been vaping. In those 3 years, the proportion of short-term vapers decreased while the proportion of longer-term vapers increased, particularly those who vape for over a year or longer. For instance, the proportion of current and former vapers who had vaped for one month or less was 16.1% in 2018 compared with 12.8% in 2021 (table 9c), and the proportion of current and former vapers who had vaped for more than 3 years was 14.5% in 2018 and 24.3% in 2021.

This changing profile of vaping duration could be explained by a steady accumulation of long-term vapers, which is supported by a comparison of current and past vapers. Among current vapers in 2021, 43.7% had vaped more than 3 years, a proportion that appears to have increased year on year, while a declining minority had vaped for 6 months or less (24.9% in 2018 versus 12.7% in 2021). Vapers who had stopped vaping are likely to have done so after a shorter period of use, although the proportion of these former vapers seems to have declined — 57.2% of past vapers in 2021 had vaped for 6 months or less (65.8% in 2018, 66.2% in 2019 and 59.9% in 2020). In comparison, only 4.8% had stopped after having vaped for more than 3 years in 2021, and this proportion has not changed much in the last 3 years.

Further research on long-term vapers should explore whether they are reducing their health risks by preventing relapse to smoking or whether, by continuing to vape, they continue to expose themselves to risks that could be avoided had they managed to quit all nicotine product use. Research on long-term vapers in England is needed including whether they are interested in stopping vaping and need support in doing so.

Table 9a. Duration of vaping among former vapers, England 2018 to 2021 (ASH-A, weighted data)

Duration	2018 (%)	2019 (%)	2020 (%)	2021 (%)
1 month or less	28.5	31.6	24.7	22.1
1 to 3 months	22.7	22.7	18.5	22.8
3 to 6 months	14.6	11.9	16.7	12.3
6 months to 1 year	13.7	10.9	16.5	15.1
1 to 2 years	9.7	8.5	9.3	13.3
2 to 3 years	2.9	4.2	5.3	5.5
More than 3 years	3.8	4.4	6.0	4.8
Don’t know	4.0	5.7	3.1	4.0

Table 9b. Duration of vaping among current vapers, England 2018 to 2021 (ASH-A, weighted data)

Duration	2018 (%)	2019 (%)	2020 (%)	2021 (%)
1 month or less	5.5	5.1	2.6	3.5
1 to 3 months	9.2	9.7	4.8	3.7
3 to 6 months	10.2	7.4	6.7	5.5
6 months to 1 year	13.0	11.3	10.9	7.6
1 to 2 years	19.9	17.5	17.1	15.5
2 to 3 years	17.9	18.4	16.9	19.2
More than 3 years	23.7	29.3	39.2	43.7
Don’t know	0.6	1.4	1.7	1.4

Table 9c. Duration of vaping among former and current vapers combined, England 2018 to 2021 (ASH-A, weighted data)

Duration	2018 (%)	2019 (%)	2020 (%)	2021 (%)
1 month or less	16.1	17.5	13.4	12.8
1 to 3 months	15.4	15.8	11.5	13.3
3 to 6 months	12.2	9.5	11.6	8.9
6 months to 1 year	13.3	11.1	13.6	11.4
1 to 2 years	15.2	13.3	13.3	14.4
2 to 3 years	11.0	11.7	11.2	12.3
More than 3 years	14.5	17.6	23.0	24.3
Don’t know	2.2	3.4	2.4	2.7

Notes: Age 18+. Unweighted bases: 2018=1,114; 2019=1,257; 2020=1,066; 2021=1,326.

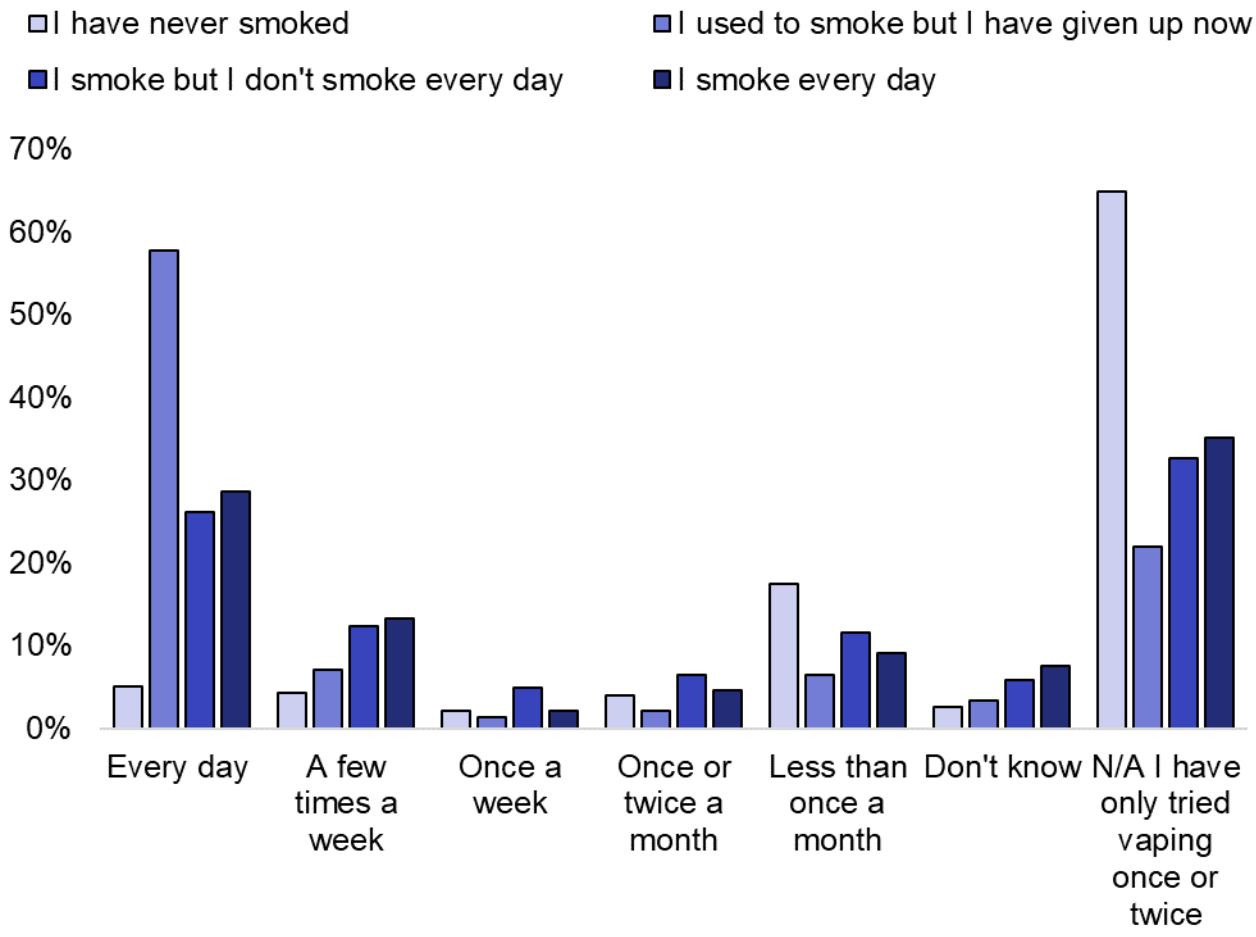
Current vaping included people who had tried vaping and who still vaped. Past vaping included people who had tried e-cigarettes but did not use them (anymore).

Vaping frequency by smoking status

The 2021 ASH-A survey recorded how often people vaped (figure 6). Among people who had ever vaped, those who had never smoked appeared to vape less frequently than

those who were current or former smokers. Nearly two-thirds (64.9%) of never smokers who had vaped had only tried vaping once or twice and 5% vaped daily. More than half (57.7%) of former smokers who had ever vaped did so every day compared with 26.1% of non-daily and 28.6% of daily smokers who had ever vaped. This is potentially because vaping is the sole source of nicotine for former smokers whereas smokers who vape consume nicotine from both smoking and vaping.

Figure 6. Vaping frequency by smoking status among adults who have ever tried vaping products, England 2021 (ASH-A, weighted data)



Notes: Age 18+. Unweighted base = 2,082.

Current smoking was split between people who smoked daily and people who smoked, but not every day. Never smoked included people who responded to a question about smoking with ‘I have never smoked’.

4.5 Smoking status of vapers

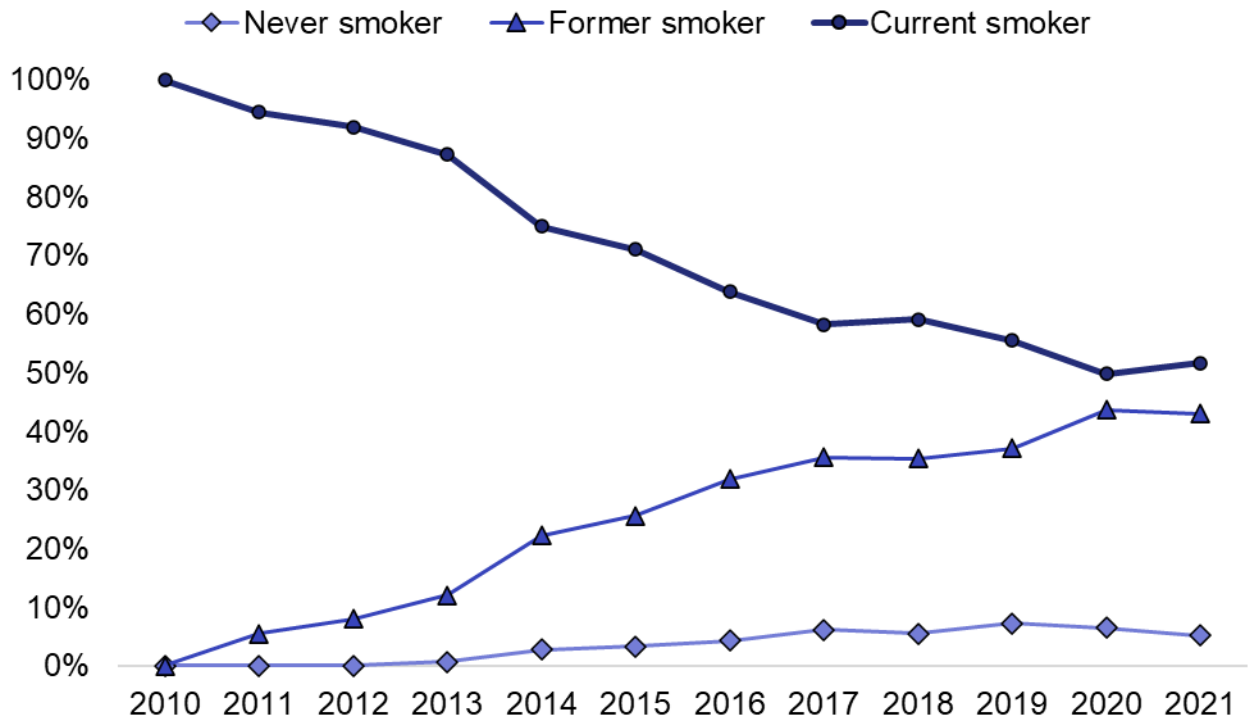
Previous figures in this chapter focused on the vaping status of current, former and never smokers. By contrast, figure 7 and figure 8 show the smoking status of current vapers in the STS and ASH-A surveys. Since 2010 (STS) and 2012 (ASH-A), current vapers have increasingly comprised former smokers with decreasing proportions of current smokers. In 2021, the decline in the proportion of vapers who currently smoke continued in the ASH-A data, but the STS proportion of vapers who also smoke slightly increased. A similar increase in the proportion of vapers who also smoke became noticeable in the ASH-A data from 2022. The 2022 data also show a 3.6 percentage point increase in the proportion of vapers who have never smoked and a 5.9 percentage point decline in the proportion of vapers who are former smokers (figure 8). These recent changes in smoking status trends among current vapers should be further monitored to explore whether former smokers are stopping vaping or returning to ‘dual use’. Similarly, the noticeably higher proportion of vapers who have never smoked in ASH-A 2022 data should be explored in future research.

The smoking status of current vapers is presented by age, region, gender, social grade and ethnicity in table 10 and table 11. Samples of vapers who had never smoked were too low in STS and ASH-A surveys from 2021 for conclusions to be drawn (table 10), but recent ASH-A data from 2022 enabled a breakdown by socio-demographics of the never smokers who vaped alongside the breakdowns of former and current smoking (table 11). The STS and ASH-A surveys in 2021 used different definitions of vaping and smoking, which made it difficult to compare findings between them. The STS survey sample comprised more vapers who were currently smoking (51.7%), while the ASH-A survey included more vapers who had stopped smoking (64.0%). Despite the discrepancy in sample compositions, there were statistically significant differences in both surveys indicating that the proportion of former smokers who vaped increased with participants’ age while the proportion of vapers who also smoked decreased with age and was highest among the youngest participants. In 2021 ASH-A data, females who vape were more often former than current smokers compared with males who vape (χ^2 (1) = 6.1, p=0.014); this difference was not identified in the 2021 STS data. No statistically significant regional or socio-economic status variations were noted in the smoking status of current vapers. The 2021 ASH-A data showed that more people from black and minority ethnic groups who vaped also smoked (47.4%) compared with 27.5% of people from white ethnic groups (χ^2 (1) = 15.7, p<0.001); the STS data did not show a statistically significant difference.

The 2022 ASH-A data indicated some changes in the smoking status profile of current vapers since 2021 (table 11). For instance, the most noticeable changes were in the proportions of current vapers who were former smokers—among 25 to 34 year olds this proportion decreased by 8.8 percentage points, among females by 8.7 percentage points, among vapers from north England by 10.4 percentage points, among vapers from C2DE social grades by 9.1 percentage points and among vapers from black and minority ethnic

groups by 15.6 percentage points. These decreases were accompanied by increases in the proportions of vapers who were also smoking—among females by 4.0 percentage points, among vapers from north England by 7.6 percentage points and among vapers from C2DE social grades by 4.9 percentage points. While these data are from 2 cross-sectional surveys conducted one year apart, it is not clear how the apparent changes in the proportions of current vapers who are former or current smokers are associated. However, these trends further reinforce the above-noted need to continue monitoring concurrent smoking and vaping trends in England.

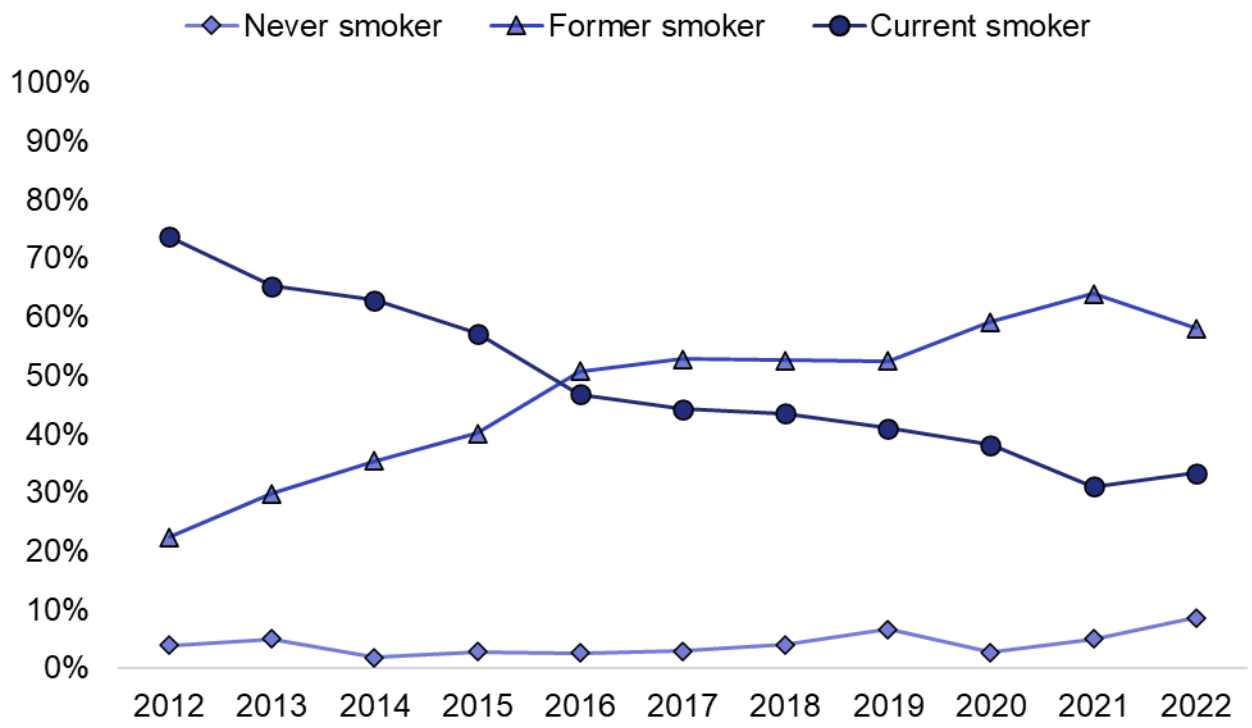
Figure 7. Smoking status of current vapers over time, England 2010 to 2021 (STS, weighted data)



Notes: Age 18+. Unweighted bases: 2010=10; 2011=116; 2012=231; 2013=747; 2014=962; 2015=1,077; 2016=1,088; 2017=1,056; 2018=1,071; 2019=1,053; 2020=1,186; 2021 (to September) =1,342. Prior to late 2013 this question had only been asked for past year smokers, so data are comparable before that date.

Current vaper included people who ‘currently vaped for any reason’. Never smoker included people who had never regularly smoked for a year or more. Former smokers included those who had stopped smoking completely but who had smoked for a year or more in the past. Current smokers included people who said that they smoked daily or that they smoked, but less than daily. Never regularly smoked included people who had never smoked for longer than one year. 2021 data available from January to September. The full year’s data were used for all other years.

Figure 8. Smoking status of current vapers over time, England 2012 to 2022 (ASH-A, weighted data)



Notes: Age 18+. Unweighted bases: 2012=180; 2013=270; 2014=407; 2015=508; 2016=545; 2017=542; 2018=620; 2019=699; 2020=564; 2021=690; 2022=901.

Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped. Never smoked included people who responded to a question about smoking with ‘I have never smoked’. Former smokers included those who said that they used to smoke, but who had “given up now”. Current smoking included people who smoked daily as well as those who smoked, but not daily.

Table 10. Smoking status of current vapers by age, gender, region, social grade and ethnicity, England 2021 (STS and ASH-A, weighted data, unweighted counts)

	STS			ASH-A		
	Never smoker % (n)	**Former Smoker % (n)**	**Current smoker % (n)**	**Never smoker* % (n)**	**Former Smoker % (n)**	**Current smoker % (n)**
Total	5.2 (50)	43.1 (412)	51.7 (480)	5.0 (31)	64.0 (446)	31.0 (213)
Age						
18 to 24	10.7 (18)	30.4 (47)	58.9 (92)	-	31.5 (17)	46.0 (26)
25 to 34	4.7 (12)	37.2 (85)	58.1 (126)	-	61.5 (68)	31.1 (37)
35 to 44	4.3 (7)	44.4 (63)	51.2 (79)	-	60.5 (97)	34.3 (53)
45 to 54	3.8 (7)	49.7 (85)	46.5 (77)	-	69.1 (91)	28.5 (39)
55 to 64	2.1 (2)	56.7 (82)	41.2 (61)	-	73.9 (173)**	25.7 (58)**
65+	5.3 (4)	50.5 (50)	44.2 (45)			
Statistical testing	-	χ^2 (5) = 23.0, p<0.001		-	χ^2 (4) = 22.4, p<0.001	
Gender						
Male	5.4 (29)	44.0 (227)	50.6 (249)	-	59.4 (218)	34.7 (126)
Female	4.9 (21)	42.0 (185)	53.1 (231)	-	69.7 (228)	26.4 (87)
Statistical testing	-	χ^2 (1) = 0.9, p=0.340		-	χ^2 (1) = 6.1, p=0.014	
Region						
North	3.6 (12)	48.0 (150)	48.5 (151)	-	67.2 (142)	27.4 (59)
Midlands	6.6 (12)	47.3 (74)	46.1 (79)	-	55.8 (85)	37.8 (53)
South	5.7 (26)	38.3 (188)	56.0 (250)	-	65.4 (219)	30.4 (101)
Statistical testing	-	χ^2 (2) = 3.5, p=0.174		-	χ^2 (2) = 3.2, p=0.199	
Social grade						
ABC1	5.7 (26)	45.3 (217)	49.0 (225)	-	59.4 (224)	33.3 (121)
C2DE	4.5 (19)	41.8 (175)	53.7 (226)	-	68.3 (222)	28.8 (92)
Statistical testing	-	χ^2 (1) = 2.6, p=0.105		-	χ^2 (1) = 2.5, p=0.114	
Ethnicity						
White	5.3 (44)	44.0 (373)	50.8 (420)	-	67.6 (392)	27.5 (162)

	STS			ASH-A		
	Never smoker % (n)	Former Smoker % (n)	Current smoker % (n)	Never smoker* % (n)	Former Smoker % (n)	Current smoker % (n)
Black and minority ethnic groups	4.2 (5)	38.7 (37)	57.1 (55)	-	48.4 (43)	47.4 (44)
Statistical testing	-	χ^2 (1) = 1.5, p=0.214		-	χ^2 (1) = 15.7, p<0.001	

Notes: STS (18+): Unweighted base for age, gender and region = 942; social grade = 888; ethnicity = 934. Current vaper included people who ‘currently vaped for any reason’. Current smoker included people who said that they smoked daily or that they smoked, but less than daily. Former smokers included those who had stopped smoking completely but who had smoked for a year or more in the past. Never smokers included people who had never regularly smoked for a year or more. STS data available from January to September 2021.

ASH-A (18+): Unweighted base for age, gender, region, social grade = 690; ethnicity = 669. Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped. Never smoked included people who responded to a question about smoking with ‘I have never smoked’. Former smokers included those who said that they used to smoke, but who had ‘given up now’. Current smoking included people who smoked daily as well as those who smoked, but not daily.

* Columns with 50 or fewer participants were not included in statistical testing as they do not represent a wide enough cross-section of the target population to be considered statistically reliable.

** The oldest age group in the ASH-A survey included participants who were 55 or older.

Table 11. Smoking status of current vapers by age, gender, region, social grade and ethnicity, England 2022 (ASH-A, weighted data, unweighted counts)

	Never smoker % (n)	Former smoker % (n)	Current smoker % (n)
Total	8.6 (89)	58.1 (504)	33.4 (308)
Age			
18 to 24	23.7 (47)	30.7 (58)	45.5 (89)
25 to 34	14.3 (23)	52.7 (83)	33.0 (53)
35 to 44	5.1 (10)	59.3 (112)	35.6 (67)
45 to 54	2.7 (4)*	67.0 (108)	30.3 (48)
55+	2.4 (5)	71.4 (143)	26.2 (51)
Statistical testing	χ^2 (8) = 117.7, p<0.001		
Gender			
Male	8.5 (44)	55.5 (250)	35.9 (173)
Female	8.6 (45)	61.0 (254)	30.4 (135)
Statistical testing	χ^2 (2) = 3.5, p=0.171		
Region			
North	8.2 (27)	56.8 (159)	35.0 (103)
Midlands	11.9 (28)	53.0 (105)	35.1 (75)
South	7.2 (34)	61.4 (240)	31.3 (130)
Statistical testing	χ^2 (4) = 6.6, p=0.162		
Social grade			
ABC1	10.2 (53)	56.8 (254)	33.0 (158)
C2DE	7.2 (36)	59.2 (250)	33.7 (150)
Statistical testing	χ^2 (2) = 2.6, p=0.279		
Ethnicity			
White	7.1 (64)	62.6 (458)	30.3 (238)
Black and minority ethnic groups	16.4 (24)	32.8 (45)	50.8 (70)
Statistical testing	χ^2 (2) = 38.2, p<0.001		

Notes: ASH-A (18+): Unweighted base for age, gender, region, social grade = 901; ethnicity = 899.

Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped. Never smoked included people who responded to a question about smoking with ‘I have never smoked’. Former smokers included those who said that they used to smoke, but who had ‘given up now’. Current smoking included people who smoked daily as well as those who smoked, but not daily.

* A cell with expected count less than 5 was included in χ^2 testing—the outcome might not be statistically reliable.

4.6 Vaping, smoking and socio-economic status

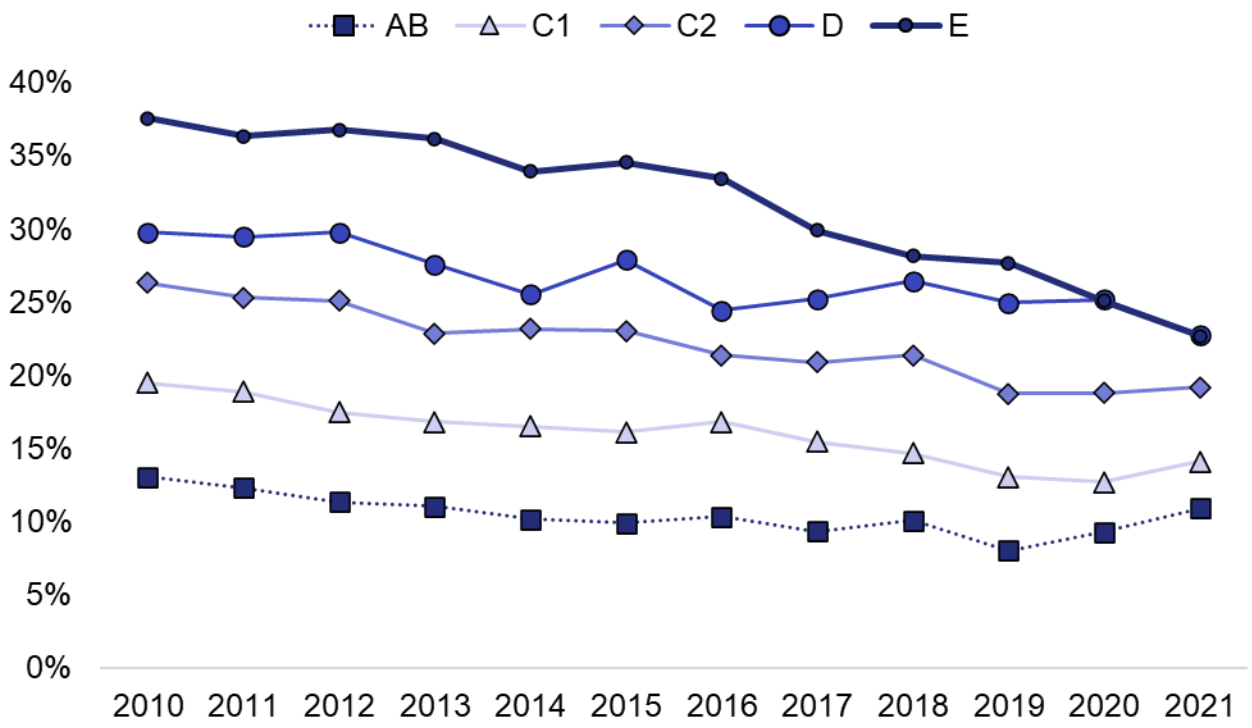
In our previous report (2) we highlighted a possible increase in smoking prevalence among people classified as ‘high or intermediate managerial, administrative or professional’ (group AB) which required further monitoring. The STS data from 2021 indicate noticeable upticks in smoking prevalence among people from the more advantaged socio-economic groups—AB (from 9.3% in 2020 to 10.9%), C1 (from 12.7% to 14.1%) and C2 (from 18.8% to 19.2%, figure 9). At the same time, smoking prevalence continued to decline among ‘semi- and unskilled manual workers’ (group D, from 25.2% in 2020 to 22.8% in 2021) and ‘pensioners, casual or lowest grade workers and unemployed’ (group E, from 25.1% in 2020 to 22.7% in 2021).

Looking back at smoking prevalence by socio-economic status for the last 5 years, there is clear evidence of a narrowing gap between smokers from the most and least advantaged socio-economic groups, mostly due to reductions in smoking among the latter. Smoking prevalence among people from social grade AB remained relatively stable (10.3% in 2016 and 10.9% in 2021), while it decreased by 10.8 percentage points among people from social grade E (from 33.5% in 2016 to 22.7% in 2021). This suggests that differences in estimates of smoking prevalence between the socio-economic status (SES) groups continue to narrow, decreasing the social inequalities of harms caused by smoking in England. However, the apparent recent small increase in smoking prevalence among people from more advantaged socio-economic groups needs to be further explored and addressed, to ensure declines in smoking prevalence occur across all socio-economic groups.

Vaping prevalence also varied between SES groups (figure 10), ranging from 4.9% in group AB to 9.1% in group D. Since 2019, increases in vaping prevalence have been observed across all SES groups, with a slightly steeper rise among C2, D and E groups. When viewing vaping prevalence among past-year smokers only (figure 11), an increase in vaping between 2020 and 2021 is noticeable across all SES groups with the steepest rise observed among smokers from the lowest SES groups—D and E.

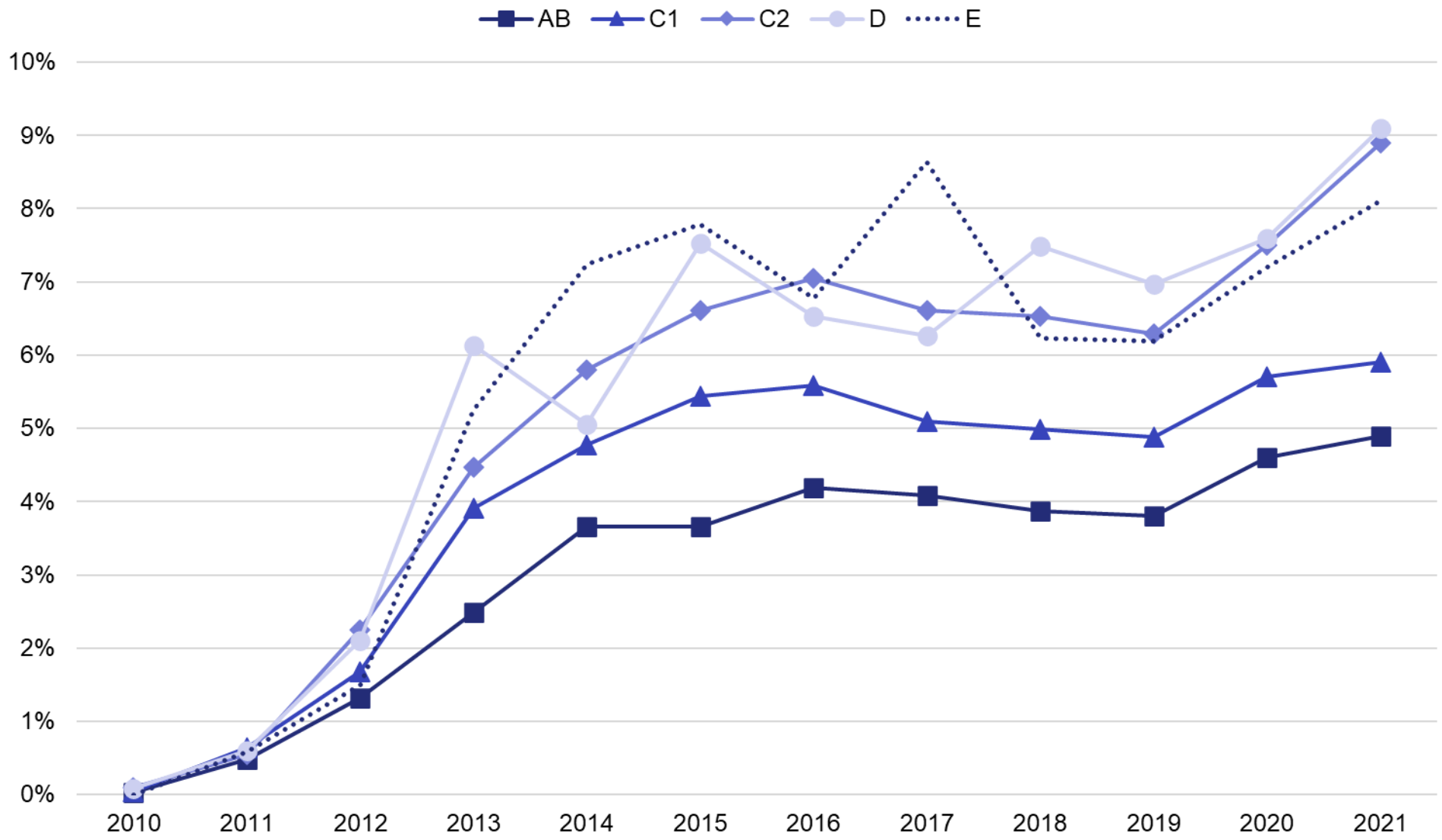
The continuing rise in vaping seems to follow the trend that has been described in a study by Kock and others using STS data (13), where the increase in vaping between 2014 and 2019 was greater among people from more disadvantaged, than more advantaged, SES groups.

Figure 9. Smoking prevalence by socio-economic status among all adults, England 2010 to 2021 (STS, weighted data)



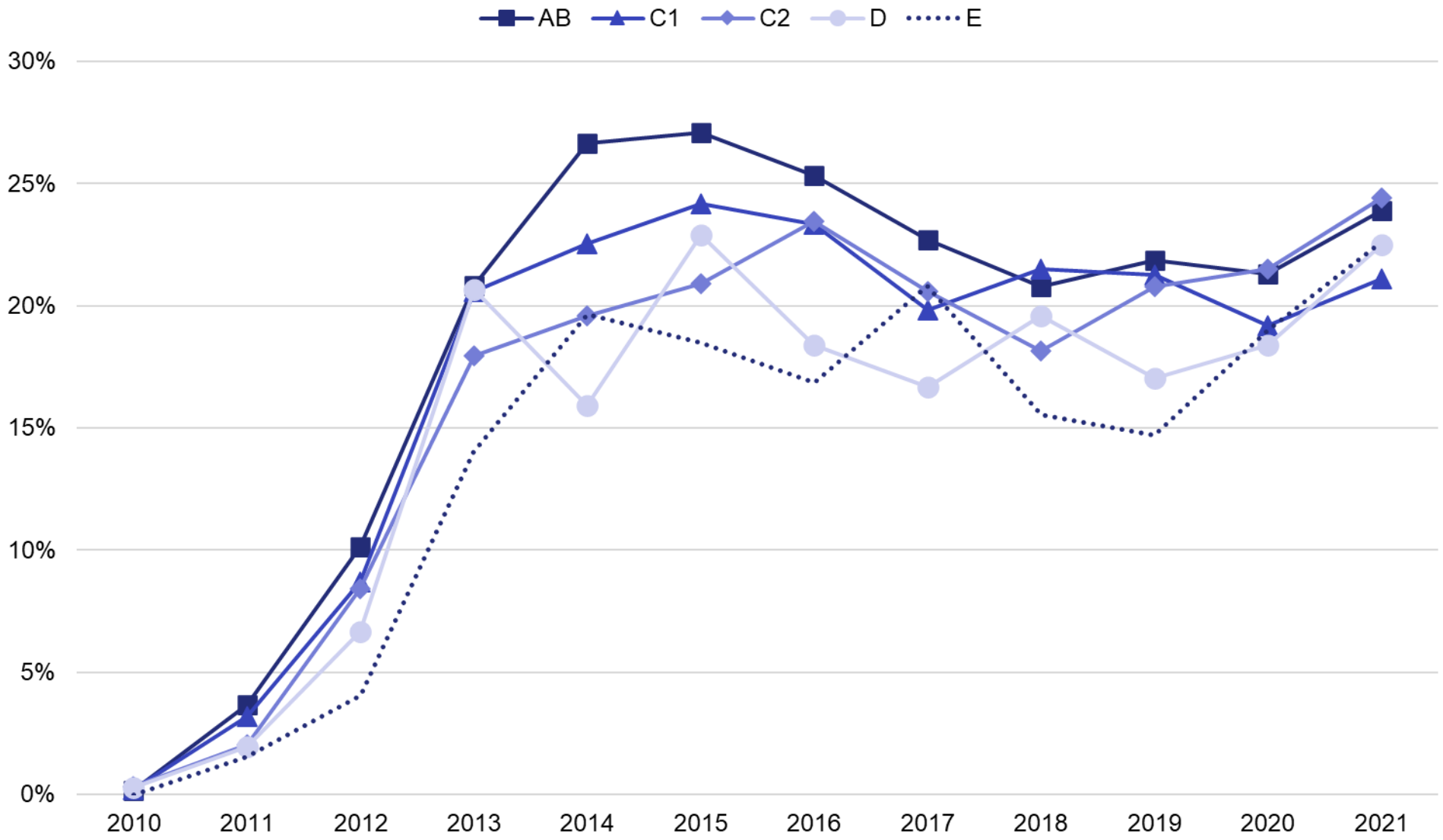
Notes: Age 18+. Unweighted bases: 2010=24,268; 2011=21,299; 2012=20,832; 2013=21,658; 2014=19,773; 2015=19,642; 2016=20,063; 2017=20,036; 2018=20,402; 2019=20,380; 2020=19,518; 2021 (January to September)=19,574. Smoking prevalence included current smokers who smoked daily or smoked, but less than daily. Social grade definitions (11): A = High managerial, administrative or professional; B = Intermediate managerial, administrative or professional; C1 = Supervisory, clerical and junior managerial, administrative or professional; C2 = Skilled manual workers; D = Semi and unskilled manual workers; E = State pensioners, casual or lowest grade workers, unemployed with state benefits only. 2021 data available from January to September. The full year's data were used for all other years.

Figure 10. Vaping prevalence by socio-economic status among all adults, England 2010 to 2021 (STS, weighted data)



Notes: Age 18+. Unweighted bases: 2010=24,294; 2011=21,315; 2012=13,897; 2013=18,311; 2014=19,798; 2015=19,650; 2016=20,066; 2017=20,051; 2018=20,421; 2019=20,385; 2020=19,518; 2021 (January to September) =19,574. Vaping prevalence included current vapers who ‘currently vaped for any reason’. Social grade definitions (11): A = High managerial, administrative or professional; B = Intermediate managerial, administrative or professional; C1 = Supervisory, clerical and junior managerial, administrative or professional; C2 = Skilled manual workers; D = Semi and unskilled manual workers; E = State pensioners, casual or lowest grade workers, unemployed with state benefits only. 2021 data available from January to September. The full year’s data were used for all other years.

Figure 11. Vaping prevalence by socio-economic status among current smokers and recent (less than one year) former smokers, England 2010 to 2021 (STS, weighted data)



Notes: Age 18+. Unweighted bases: 2010=6,005; 2011=5,191; 2012=3,360; 2013=3,936; 2014=4,203; 2015=4,147; 2016=3,922; 2017=3,651; 2018=3,755; 2019=3,408; 2020=3,218; 2021 (January to September) =3,378. Smokers included people who smoked daily as well as those who smoked, but not daily. Recent former smokers included those who had stopped smoking completely, who had previously smoked for a year or more and who quit smoking under one year ago. Vaping prevalence included current vapers who ‘currently vaped for any reason’. Social grade definitions (11): A = High managerial, administrative or professional; B = Intermediate managerial, administrative or professional; C1 = Supervisory, clerical and junior managerial, administrative or professional; C2 = Skilled manual workers; D = Semi and unskilled manual workers; E = State pensioners, casual or lowest grade workers, unemployed with state benefits only. 2021 data available from January to September. The full year’s data were used for all other years.

4.7 Reasons for vaping

The 2021 ASH-A survey asked current vapers for the single main reason why they used a vaping product. These reasons, also split between former or current smokers, are provided in table 12.

Three out of the four most popular reasons for vaping among current vapers were related to smoking cessation or reduction. However, this might be due to the higher proportion of former (64.6%) than current (35.4%) smokers in the sample. More than half of former smokers vaped to help them give up smoking entirely (35.2%) or to help them keep off tobacco (19.6%, table 12). This indicates that many former smokers continue to use vaping products to prevent relapse. The main reason endorsed by about a quarter (26.2%) of current smokers who vaped was to help them reduce smoking but not stop completely, which also suggests use of vaping products for tobacco harm reduction. The most common reason selected by never smokers was ‘because I enjoy the experience’ (40.1%, n=12), but the small number of never smokers who vaped (n=31) prevents reliable estimates being drawn.

There was some variation in the main reason for vaping by sociodemographic characteristics (data not shown). When broken down by age, the largest proportion of participants who reported vaping to help them stop smoking was among 25 to 34 year old former smokers (52.8%), and 35 to 44 year old current smokers were the group who most frequently reported vaping to reduce the amount of smoking, but not stop completely (36.4%). There were no apparent differences in the main reason for vaping between male and female vapers. Compared with vapers who smoke from ABC1 SES groups, slightly more vapers who smoke from C2DE groups reported vaping to stop smoking (12.1% and 21.5% respectively) and to keep off tobacco (11.5% and 19.6%), while more current smokers from ABC1 groups reported vaping to reduce their smoking, but not stop completely (31.7% and 20.2%).

Table 12. Main reason for vaping among current vapers, England 2021 (ASH-A, weighted data, unweighted counts)

Reason	Former smoker % (n)	Current smoker % (n)	Total % (n)
To help me stop smoking tobacco entirely*	35.2 (152)	16.6 (32)	27.9 (185)
I want an aid to help me keep off tobacco*	19.6 (94)	15.4 (34)	17.7 (130)
Because I enjoy the experience	13.1 (59)	7.0 (16)	12.6 (87)
To help me reduce the amount of tobacco I smoke, but not stop completely*	3.2 (14)	26.2 (56)	10.4 (71)
To save money compared with smoking tobacco	10.8 (47)	8.6 (19)	9.6 (66)
Because I feel I am addicted to smoking tobacco and cannot stop using it even though I want to*	7.4 (31)	4.7 (10)	6.2 (14)
Just to give it a try	1.8 (8)	4.7 (10)	3.4 (23)
I need something to help deal with situations where I cannot smoke (for example workplaces, bars or restaurants)	0.7 (4)	7.5 (16)	2.8 (20)
To avoid putting those around me at risk due to second-hand tobacco smoke	2.5 (11)	3.0 (6)	2.5 (17)
It was suggested or recommended by a friend	0.9 (4)	3.1 (6)	2.0 (13)
It was advised by a health professional*	1.2 (6)	0.8 (2)	1.1 (9)
Other	3.6 (16)	2.5 (6)	3.8 (28)

Notes: Age 18+. Unweighted base = 690. Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped. Former smokers included those who said that they used to smoke, but who had ‘given up now’. Current smoking included people who smoked daily as well as those who smoked, but not daily. The reasons given by never smokers are not shown because the number (n=31) does not represent a wide enough cross-section of the target population to be considered statistically reliable. Nevertheless, total unweighted counts include counts of never smokers.

* Indicates reasons to vape that are related to smoking cessation or reduction.

4.8 Vaping products

Rechargeable models with tanks designed to be refilled by the user (tank models) remained the most popular type of vaping product (STS, figure 12) with an estimated 59.3% of current vapers using this type of device in 2021. Also, in the STS survey, modular models (which are tank models where vapers use their own combination of device parts) were used by 20.1% of vapers, cartridge models by 14.9% and disposable models by 4.6%. This order of preference was also reflected in the 2021 and 2022 ASH-A data, with tank models being most popular among both former and current vapers. However, the ASH-A data indicate a marked increase in the use of disposable models among current and former vapers in 2022 (figure 13). When viewed over time until 2021 (figure 14), vaping product preferences had remained relatively stable with a small change after 2019 away from modular devices (5.5 percentage point decrease in prevalence over the last 2 years) and towards tank devices (7.3 percentage point increase in prevalence over the last 2 years). Nevertheless, the recently reported vaping device preferences in the 2022 ASH-A survey indicate a rising popularity of the new generation of disposable vaping devices.

Table 13 shows the vaping product preferences across sociodemographic characteristics between STS and ASH-A data from 2021 and table 14 shows vaping product preferences in 2022 ASH-A data. The STS survey in 2021 showed statistically significant differences when vaping device preferences were compared by age with cartridge devices more popular among older age groups (for example, 28.2% among those aged 65 and over), and tank devices more popular among younger age groups (for example, 66.5% among those aged 18 to 24). Similar differences were not detected in the 2021 ASH-A survey. According to the ASH-A data from 2021, a higher proportion of current vapers from C2DE socio-economic groups were using tank devices (79.4%) compared with those from ABC1 socio-economic groups, and a higher proportion of current vapers from ABC1 groups using cartridges compared to those in C2DE groups (72.5%, χ^2 (1) = 5.9, p=0.015); a similar, but not statistically significant trend could be inferred from the STS data. There were also statistically significant differences between vapers of different ethnic background and of different smoking status in ASH-A survey, but these were not substantiated by the STS data (table 13).

The overall increase in disposable product use in the 2022 ASH-A survey was most noticeable among 18 to 24 and 25 to 34 year old participants (46.3% and 20.1% of vapers within these age groups respectively used disposable devices). A recent paper by Tattan-Birch and others (14) among STS participants surveyed between January 2021 and January 2022 found a 14-fold increase in the percentage of vapers that used disposable products, with the increase being most pronounced among young adults. However, they also found that overall prevalence of inhaled nicotine, either vaping or smoking, remained stable, particularly in young adults.

In the ASH-A data, although there was a substantial decrease in the proportion of tank type vaping product users between 2021 and 2022 (from 76.1% to 64.3%), these products remained the most popular among all current vapers, most commonly used by former smokers (77.1%; table 14). While the numbers are small, the proportions of never smokers and black and minority ethnic groups using disposable vaping products in the 2022 ASH-A survey merits further research (table 14).

Figure 12. Type of vaping product used by current (STS) and current and former vapers (ASH-A), England 2021 (weighted data)

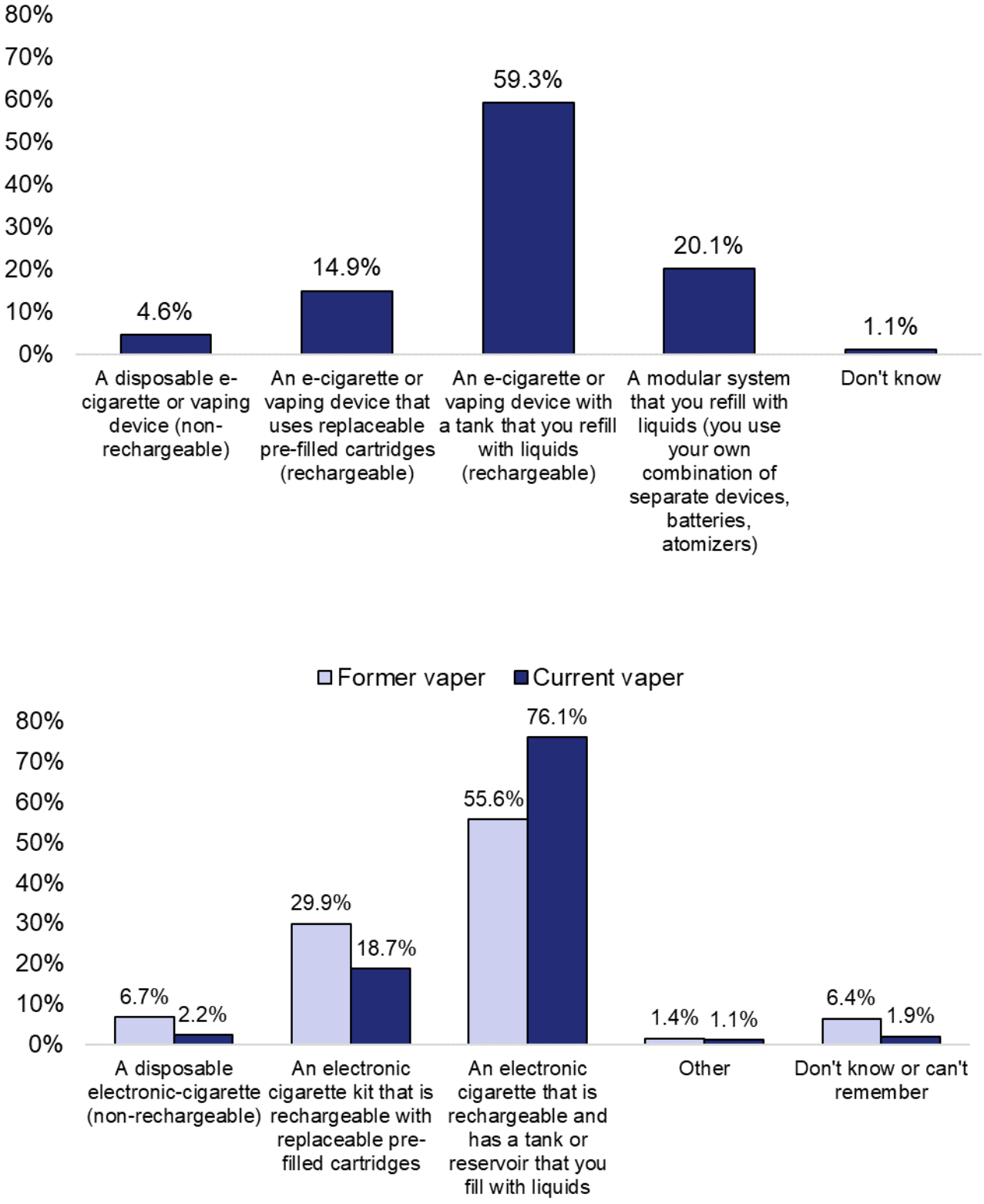
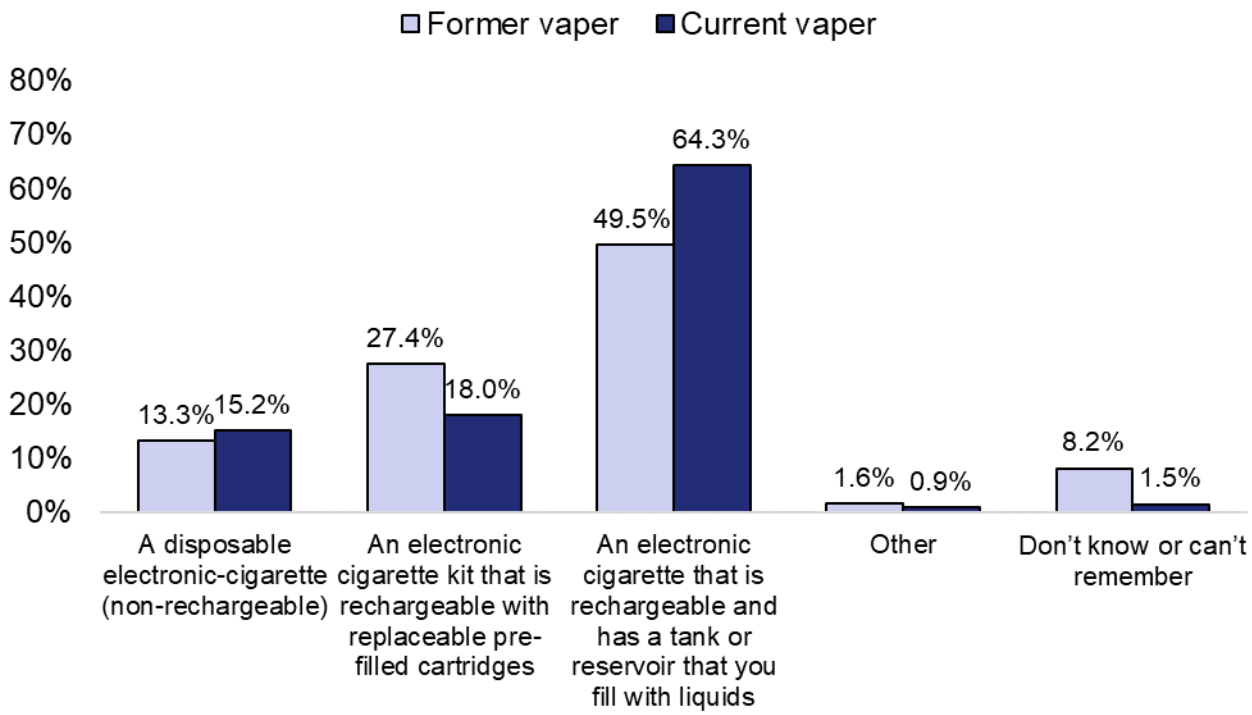


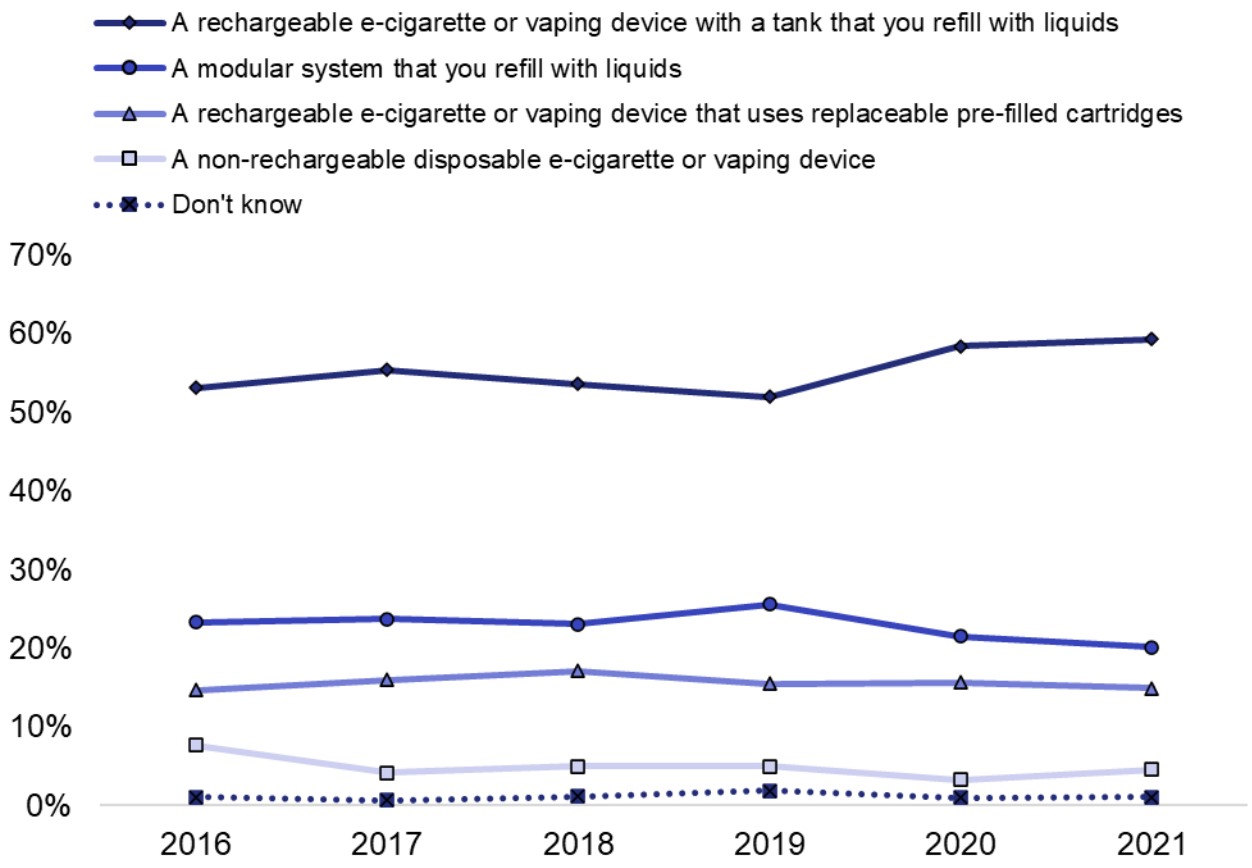
Figure 13. Type of vaping product used by current and former vapers, England 2022 (ASH-A; weighted data)



Notes: STS: Age 18+. Unweighted base (January to September 2021) = 1,209. Current vaper included people who 'currently vaped for any reason'.

ASH-A: Age 18+. Unweighted bases: 2021=1326 (current vaping=660, former vaping=666); 2022=1622 (current vaping=850, former vaping=772). Current vaping included people who had tried vaping and who still vaped. Former vaping included people who had tried e-cigarettes but did not use them (anymore).

Figure 14. Type of vaping product used by current vapers, England 2016 to 2021 (STS, weighted data)



Notes: Age 18+. Unweighted bases: 2016=490; 2017=1,044; 2018=1,040; 2019=980; 2020=1,050; 2021 (to September) =1,209. Current vapers included people who ‘currently vaped for any reason. 2021 data available from January to September. The full year’s data were used for all other years.

Table 13. Type of vaping products used by current vapers by age, gender, region social grade and ethnicity, England 2021 (STS and ASH-A, weighted percentage, unweighted counts)

	STS	STS	STS	STS		ASH-A	ASH-A	ASH-A
	Disposable* % (n)	Cartridge % (n)	Tank % (n)	Modular % (n)		Disposable* % (n)	Cartridge % (n)	Tank % (n)
Total	4.6 (34)	14.9 (134)	59.3 (496)	20.1 (174)		2.2 (15)	18.7 (125)	76.1 (505)
Age					**Age**			
18 to 24	-	12.3 (18)	66.5 (83)	12.5 (19)	18 to 24	-	33.3 (16)	58.5 (27)
25 to 34	-	7.9 (18)	61.1 (119)	21.9 (45)	25 to 34	-	18.9 (21)	74.1 (80)
35 to 44	-	14.9 (20)	56.6 (80)	24.9 (33)	35 to 44	-	18.1 (27)	73.2 (113)
45 to 54	-	17.4 (24)	58.8 (92)	21.7 (35)	45 to 54	-	16.3 (23)	81.1 (105)
55 to 64	-	20.3 (26)	56.9 (74)	22.2 (29)	55+	-	17.1 (38)	80.6 (180)
65+	-	28.2 (28)	51.6 (48)	12.2 (12)				
Statistical testing	-	χ^2 (10) = 26.5, p=0.003				-	χ^2 (4) = 9.3, p=0.054	
Gender								
Male	-	12.9 (61)	61.5 (280)	21.0 (95)		-	17.6 (59)	76.7 (267)
Female	-	17.5 (73)	56.6 (216)	19.0 (79)		-	20.0 (66)	75.3 (238)
Statistical testing		χ^2 (2) = 5.1, p=0.079				-	χ^2 (1) = 1.3, p=0.256	
Region								
North	-	11.5 (39)	64.0 (190)	21.4 (63)		-	15.2 (31)	80.9 (161)
Midlands	-	12.7 (19)	63.3 (91)	16.3 (24)		-	21.8 (28)	74.2 (107)
South	-	18.5 (76)	54.1 (215)	20.7 (87)		-	19.5 (66)	73.9 (237)
Statistical testing	-	χ^2 (4) = 9.1, p=0.059				-	χ^2 (2) = 2.4, p=0.296	
Social grade								
ABC1	-	16.2 (68)	57.3 (242)	21.1 (95)		-	22.6 (79)	72.5 (258)
C2DE	-	13.9 (58)	60.9 (226)	19.2 (68)		-	14.9 (46)	79.4 (247)
Statistical testing	-	χ^2 (2) = 2.1, p=0.348				-	χ^2 (1) = 5.9, p=0.015	
Ethnicity								
White	-	15.1 (120)	59.7 (444)	20.4 (158)		-	16.9 (98)	79.2 (439)
Black and minority ethnic groups	-	13.4 (12)	57.5 (48)	18.5 (15)		-	28.4 (24)	58.0 (49)

	STS	STS	STS	STS		ASH-A	ASH-A	ASH-A
	Disposable* % (n)	**Cartridge % (n)**	**Tank % (n)**	**Modular % (n)**		**Disposable* % (n)**	**Cartridge % (n)**	**Tank % (n)**
Statistical testing	-	χ^2 (2) = 0.2, p=0.907				-	χ^2 (1) = 8.6, p=0.003	
Smoking status								
Never smoker	-	16.2 (7)	61.5 (27)	16.6 (8)		-	10.5 (2)**	81.9 (13)
Former smoker	-	11.8 (47)	64.6 (235)	21.3 (80)		-	13.4 (62)	83.7 (369)
Current smoker	-	17.5 (80)	54.6 (234)	19.4 (86)		-	30.5 (61)	59.4 (123)
Statistical testing	-	χ^2 (4) = 7.1, p=0.131				-	χ^2 (2) = 29.0, p<0.001**	

Notes: STS (18+): Unweighted base for age, gender and region = 848; social grade = 799; ethnicity = 840. Ten participants said that they did not know which type of vaping device they used. Current vaper included people who ‘currently vaped for any reason’. STS data available from January to September 2021.

ASH-A (18+): Unweighted base for age, gender, region and social grade = 660; ethnicity = 640. Current vaping included people who had tried vaping and who still vaped. Five participants (1.1%) said that they most often used other type of vaping device and 10 participants (1.9%) said that they did not know or could not remember which type of vaping device they used.

* Columns with fewer than 50 participants have not been broken down by socio-demographic characteristics and were not included in statistical testing as they do not represent a wide enough cross-section of the target population to be considered statistically reliable.

** A cell with expected count less than 5 was included in χ^2 testing—the outcome might not be statistically reliable.

Table 14. Type of vaping products used by current vapers by age, gender, region social grade and ethnicity, England 2022 (ASH-A, weighted percentage, unweighted counts)

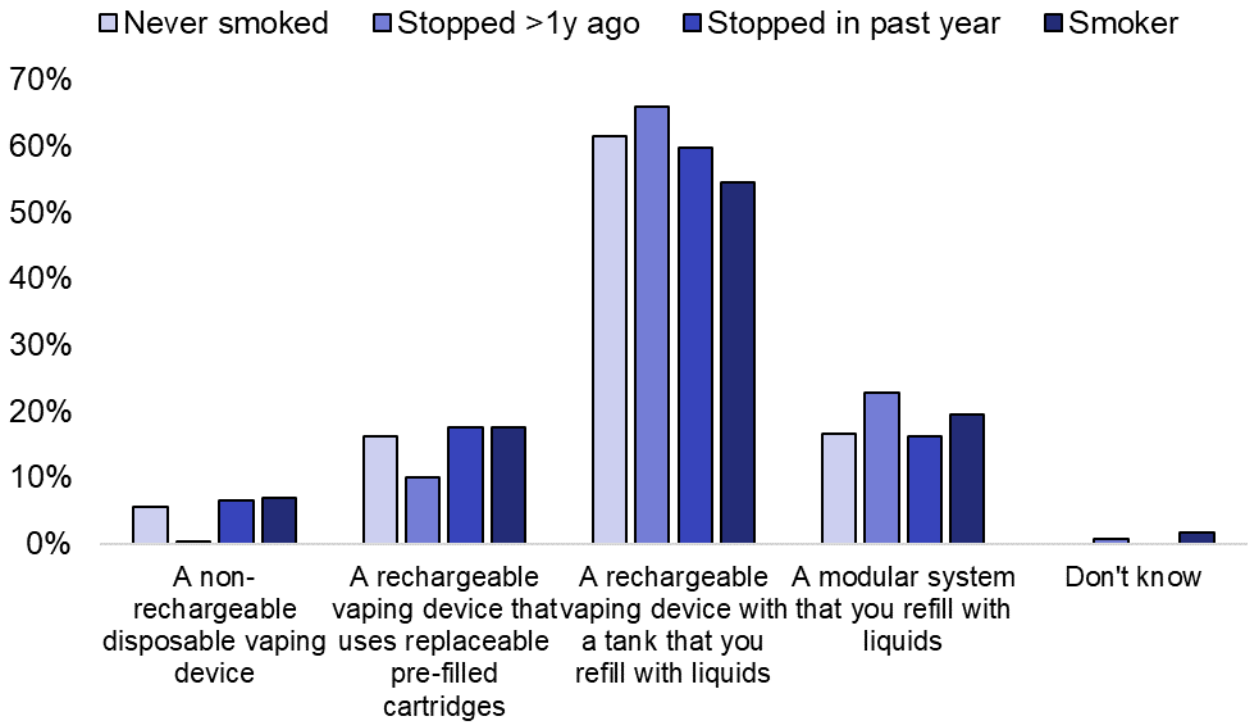
	Disposable, % (n)	Cartridge, % (n)	Tank, % (n)
Total	15.2 (152)	18.0 (152)	64.3 (524)
Age			
18 to 24	46.3 (86)	19.2 (33)	30.3 (53)
25 to 34	20.1 (29)	17.9 (27)	59.8 (84)
35 to 44	10.3 (18)	18.4 (33)	67.6 (122)
45 to 54	3.8 (6)	17.6 (27)	77.2 (122)
55+	6.5 (13)	17.3 (32)	75.1 (143
Statistical testing	χ^2 (8) = 172.8, p<0.001		
Gender			
Male	12.4 (64)	18.3 (82)	66.7 (285)
Female	18.5 (88)	17.6 (70)	61.7 (239)
Statistical testing	χ^2 (2) = 7.4, p=0.025		
Region			
North	13.2 (41)	16.7 (44)	67.9 (179)
Midlands	19.0 (45)	17.3 (34)	60.4 (109)
South	14.8 (66)	19.2 (74)	63.7 (236)
Statistical testing	χ^2 (4) = 7.0, p=0.139		
Social grade			
ABC1	17.9 (94)	17.4 (78)	61.6 (262)
C2DE	12.7 (58)	18.5 (74)	66.9 (262)
Statistical testing	χ^2 (2) = 6.7, p=0.035		
Ethnicity			
White	14.0 (121)	17.3 (124)	66.8 (467)
Black and minority ethnic groups	22.7 (31)	22.6 (28)	49.1 (56)
Statistical testing	χ^2 (2) = 12.4, p=0.002		
Smoking status			
Never smoker	38.1 (24)	20.9 (12)	38.8 (21)
Former smoker	8.9 (52)	13.0 (63)	77.1 (376)
Current smoker	22.8 (76)	26.4 (77)	45.8 (127)
Statistical testing	χ^2 (4) = 100.8, p<0.001		

Notes: ASH-A (18+): Unweighted base = 850. Current vaping included people who had tried vaping and who still vaped. Eight participants (0.9%) said that they most often used an ‘other’ type of vaping device and 14 participants (1.5%) said that they did not know or could not remember which type of vaping device they used.

In the STS, there were only small differences between never smokers, short term former (stopped in the past year), long-term former (stopped more than a year ago) and current smokers in their choice of vaping products (figure 15).

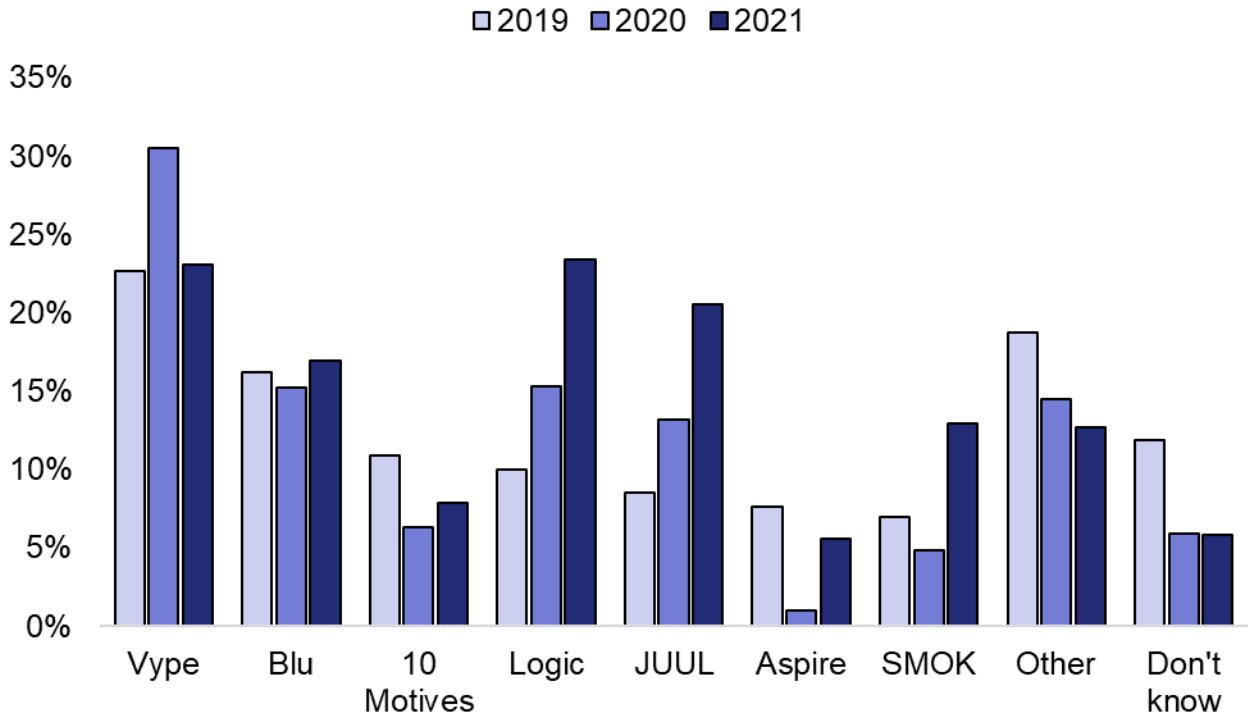
The 2021 ASH-A survey questioned vapers about which brand of vaping device they used. Logic (23.4%), Vype (23.1%) and JUUL (20.5%) were the most popular vaping products used by current vapers in 2021 (figure 16). However, Vype (Vuse from May 2021) use seemed to decline compared with 2020, while use of Logic and JUUL increased year-on-year since 2019. The numbers for years 2019, 2020 and 2021 in this figure are low (n=151, n=104 and n=157, respectively) and should be treated with caution.

Figure 15. Type of vaping product by smoking status among current vapers, England 2021 (STS, weighted data)



Notes: Age 18+. Unweighted base = 848. Current vaper included people who ‘currently vaped for any reason’. Never smoker included people who had never smoked for longer than one year. Former smokers (who stopped over or under one year ago) included those who had stopped smoking completely but who had smoked for a year or more in the past. Current smoker included people who said that they smoked daily or that they smoked, but less than daily. STS data available from January to September 2021.

Figure 16. Brand of vaping product used by current vapers by year, England 2019 to 2021 (ASH-A, weighted data)



Age 18+. Unweighted bases = 2019=151; 2020=104; 2021=157. Current vapers included people who had tried vaping and who still vaped, excluding those who no longer vaped. Vaping product brands that were reported by fewer than 10 participants in 2021 were excluded from the figure. Vype changed name to Vuse in May 2021, after data collection for ASH-A.

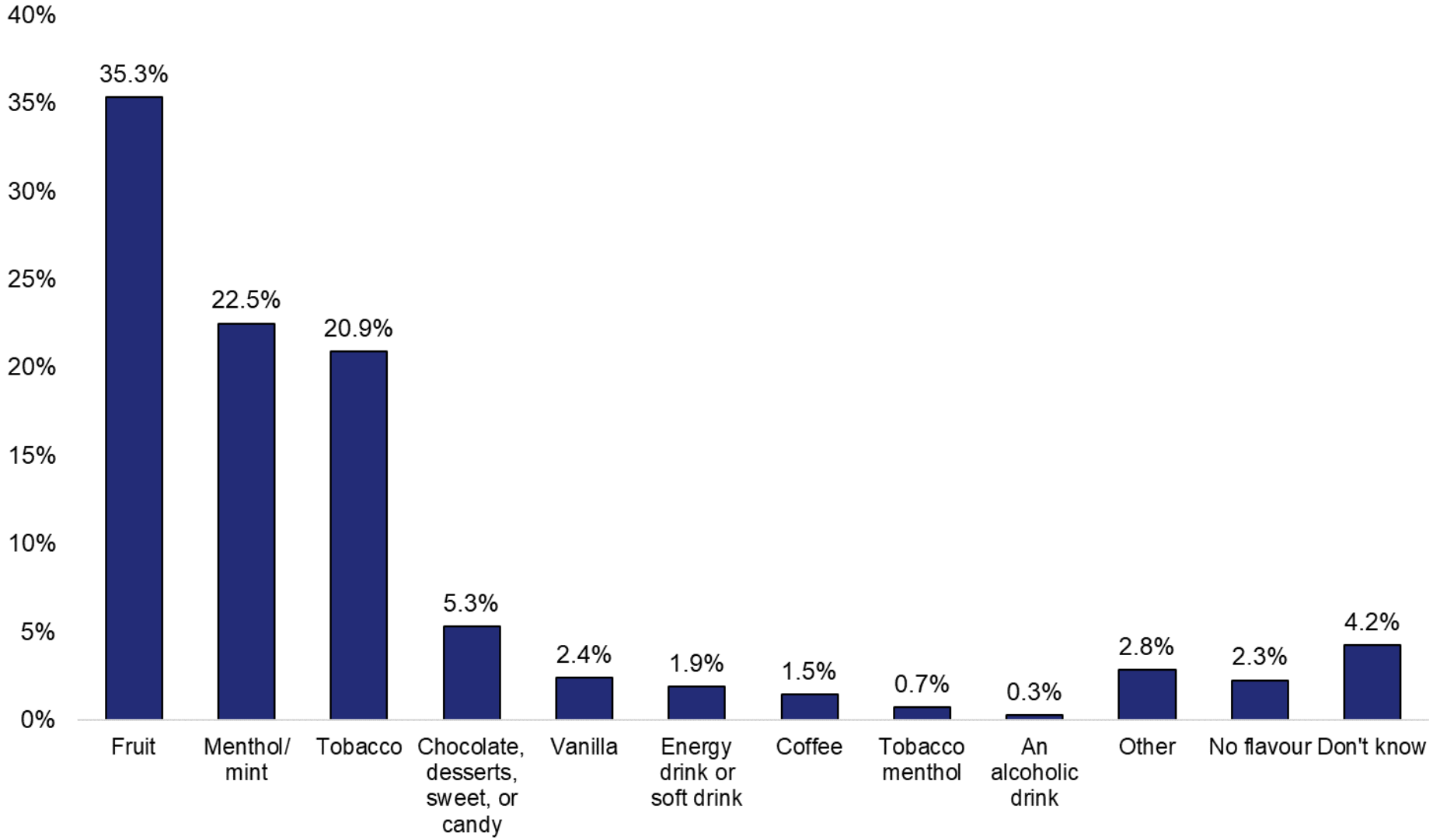
4.9 Flavours

The 2021 ASH-A survey asked people who vaped about the flavour they used most often. Fruit flavours were the main flavour for 35.3% of current vapers, menthol/mint flavours for 22.5% and tobacco flavours for 20.9%. All other flavours were used by less than 10% of current vapers—a pattern which aligns with our previous report (2).

When broken down by socio-demographics (table 15), tobacco flavours seemed to be preferred by older vapers, with 35.6% of vapers aged over 55 using tobacco-flavoured vaping liquids. Among younger vapers, around half of 18 to 24 year olds (49.7%) and 25 to 34 year olds (50.5%) preferred fruit flavours. More female vapers (27.4%) preferred menthol/mint flavours than male vapers (18.6%), but the difference was not statistically significant (χ^2 (2) = 6.0, p=0.051). There were no discernible differences in flavour preferences by region, social grade or ethnicity. Despite the small sample size, it is worth

noting that never smokers who vaped almost exclusively reported fruit flavours as their preferred flavour.

Figure 17. Flavour preferences among adults who currently vape, England 2021 (ASH-A, weighted data)



Notes: Age 18+. Unweighted base = 690. Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped. Respondents selected the one flavour they used most often.

Table 15. Flavour preferences among adults who currently vape, England 2021 (ASH-A, weighted percentage, unweighted counts)

	Fruit % (n)	Tobacco % (n)	Menthol/mint % (n)
Total	35.3 (237)	20.9 (153)	22.5 (158)
Age			
18 to 24	49.7 (28)	4.0 (2)	14.0 (7)
25 to 34	50.5 (52)	5.7 (7)	24.6 (31)
35 to 44	32.5 (53)	17.5 (30)	23.4 (37)
45 to 54	38.2 (50)	23.9 (31)	22.6 (32)
55+	22.8 (54)	35.6 (83)	22.6 (51)
Statistical testing	χ^2 (8) = 63.1, p<0.001		
Gender			
Male	34.5 (117)	21.7 (87)	18.6 (68)
Female	36.4 (120)	19.8 (66)	27.4 (90)
Statistical testing	χ^2 (2) = 6.0, p=0.051		
Region			
North	38.2 (76)	19.0 (44)	22.1 (47)
Midlands	34.4 (49)	16.2 (25)	26.8 (42)
South	33.9 112)	24.0 (84)	20.9 (69)
Statistical testing	χ^2 (4) = 6.4, p=0.171		
Social grade			
ABC1	35.9 (129)	19.4 (79)	23.3 (83)
C2DE	34.7 (108)	22.3 (74)	21.8 (75)
Statistical testing	χ^2 (2) = 0.3, p=0.852		
Ethnicity			
White	35.0 (199)	21.5 (132)	23.9 (139)
Black and minority ethnic groups	37.6 (31)	16.7 (16)	16.1 (15)
Statistical testing	χ^2 (2) = 1.4, p=0.498		
Smoking status			
Never smoker	36.2 (12)	0.0 (0)*	2.9 (1)*
Former smoker	36.4 (156)	21.7 (105)	25.0 (113)
Current smoker	32.9 (69)	22.5 (48)	20.5 (44)
Statistical testing	χ^2 (4) = 13.7, p=0.008*		

Notes: Age 18+. Unweighted base for age, gender, region and social grade = 690; ethnicity = 669. Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped.

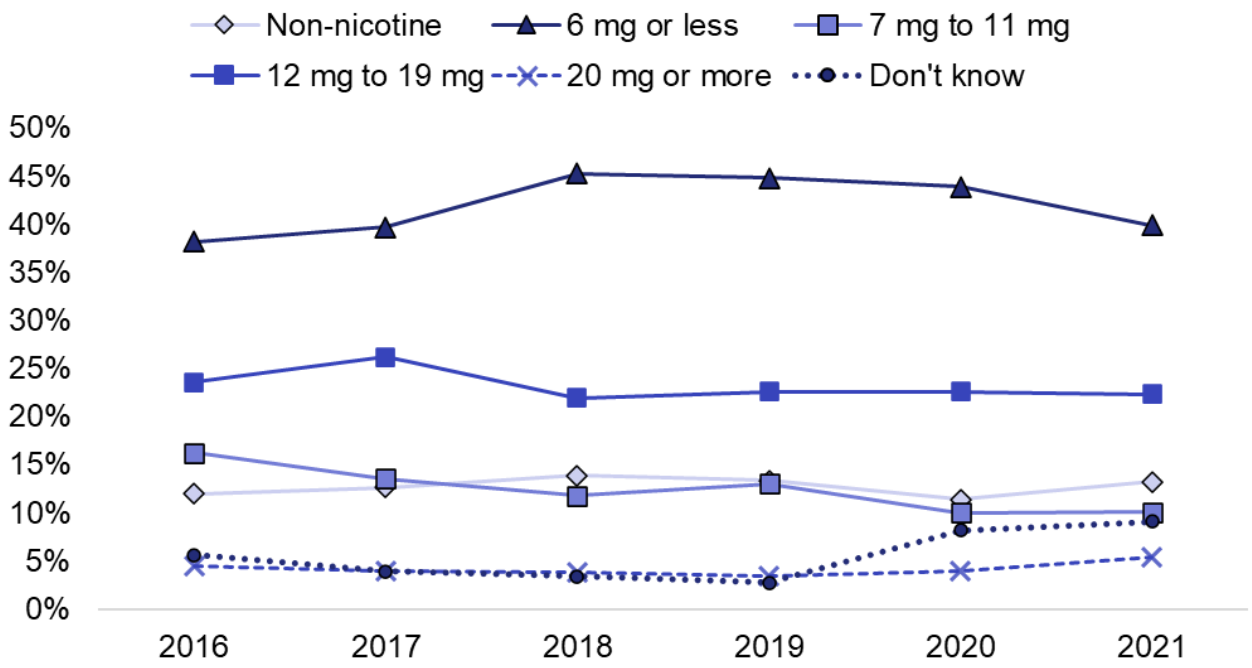
* Two cells with expected count less than 5 were included in χ^2 testing—the outcome might not be statistically reliable.

4.10 Nicotine

Since 2016, the most popular strength of vaping liquid has been 6 milligrams per millilitre (mg/mL) or less—38.2% in 2016 and 39.9% in 2021 (STS - figure 18). In 2021, 85.5% of current vapers used nicotine products below 20mg/mL and 5.4% used products with nicotine strength of 20mg/mL or stronger. Vaping liquids with nicotine strength over 20mg/mL are not allowed on the market in the UK, but STS data do not discern between vapers using 20mg/mL strength and those using vaping liquids stronger than this, which are not legally permitted. The proportion of vapers who did not know the nicotine strength of their vaping liquids appears to have increased in the last few years—from 2.7% in 2019 to 8.2% in 2020 and 9.1% in 2021, with a higher proportion of those who did not know the strength of their vaping liquid among older vapers (table 16). The ITC youth survey data indicated a decrease in ‘don’t know’ responses between 2019 and 2021 among young people who vaped (chapter 3).

Non-nicotine liquids were used by 13.2% of vapers across different age groups. An estimated 17.1% of women used non-nicotine vaping liquids compared with 10.0% of men, and it was more common among vapers from C2DE groups (15.2%) than from ABC1 groups (10.3%) (STS - table 16). Differences in nicotine strength by ethnicity and smoking status should be interpreted with caution due to the low numbers available (table 16).

Figure 18. Nicotine strength by year among current vapers, England 2016 to 2021 (STS, weighted data)



Notes: Age 18+. Unweighted bases: 2016=490; 2017=1,044; 2018=1,040; 2019=980; 2020=1,050; 2021 (January to September) =1,209. Current vapers included people who ‘currently vaped for any reason’. 2021 data available from January to September. The full year’s data were used for all other years.

Table 16. Nicotine strength used by current vapers by age, gender, region, social grade and ethnicity, England 2021 (STS, weighted data, unweighted counts)

	Non-nicotine % (n)	6mg or less % (n)	7mg to 11mg % (n)	12mg to 19mg % (n)	20mg or more* % (n)	Don't know % (n)
Total	13.2 (112)	39.9 (322)	10.1 (85)	22.3 (200)	5.4 (44)	9.1 (85)
Age						
18 to 24	15.6 (21)	42.4 (50)	4.6 (7)	25.5 (36)	-	4.9 (8)
25 to 34	11.1 (23)	50.3 (101)	9.6 (18)	16.1 (32)	-	6.3 (12)
35 to 44	13.1 (19)	39.2 (54)	11.4 (16)	23.5 (30)	-	6.0 (10)
45 to 54	12.8 (18)	40.2 (60)	8.0 (14)	24.2 (40)	-	12.5 (18)
55 to 64	13.6 (18)	31.7 (39)	15.7 (17)	24.0 (34)	-	11.8 (18)
65+	15.3 (13)	17.6 (18)	14.1 (13)	27.5 (28)	-	20.5 (19)
Statistical testing	χ^2 (20) = 53.1, p<0.001					
Gender						
Male	10.0 (48)	44.4 (185)	10.5 (49)	21.6 (108)	-	7.9 (40)
Female	17.1 (64)	34.3 (137)	9.6 (36)	23.1 (92)	-	10.7 (45)
Statistical testing	χ^2 (4) = 9.1, p=0.058					
Region						
North	13.7 (40)	35.2 (98)	12.6 (35)	25.8 (87)	-	7.4 (27)
Midlands	17.9 (29)	40.8 (55)	5.6 (8)	19.3 (26)	-	6.1 (12)
South	10.9 (43)	43.1 (169)	10.0 (42)	20.8 (87)	-	11.7 (46)
Statistical testing	χ^2 (8) = 22.6, p=0.004					
Social grade						
ABC1	10.3 (45)	42.5 (174)	9.6 (41)	22.9 (100)	-	8.9 (43)
C2DE	15.2 (61)	38.7 (134)	10.3 (38)	21.6 (85)	-	8.9 (35)
Statistical testing	χ^2 (4) = 6.5, p=0.166					
Ethnicity						
White	13.0 (98)	39.2 (281)	11.4 (83)	23.3 (185)	-	8.2 (72)
Black and minority ethnic groups	13.1 (12)	46.9 (40)	1.1 (1)**	14.8 (14)	-	14.4 (10)
Statistical testing	χ^2 (4) = 12.1, p=0.016					

	Non-nicotine % (n)	6mg or less % (n)	7mg to 11mg % (n)	12mg to 19mg % (n)	20mg or more* % (n)	Don't know % (n)
Smoking status						
Never smoker	16.4 (8)	33.1 (14)	9.5 (5)**	27.8 (11)	-	10.1 (5)**
Former smoker	13.8 (50)	43.0 (152)	12.2 (41)	23.4 (96)	-	3.9 (18)
Current smoker	12.3 (54)	37.9 (156)	8.4 (39)	20.8 (93)	-	13.5 (62)
Statistical testing	χ^2 (8) = 23.0, p=0.003					

Notes: Age 18+. Unweighted base for age, gender, region and smoking status = 848; Social grade = 799; Ethnicity = 840. Current vapers included people who 'currently vaped for any reason'. STS data available from January to September 2021.

* Columns with fewer than 50 participants have not been broken down by socio-demographic characteristics as they do not represent a wide enough cross-section of the target population to be considered statistically reliable; for this reason, data for vapers using 20mg or more were not included in statistical testing.

** Cells with expected count less than 5 were included in χ^2 testing—the outcome might not be statistically reliable.

The 2021 ASH-A data suggest that most current vapers had either reduced (34.0%) or continued using the same (31.4%) strength of nicotine liquid since they started to vape (table 17). A quarter of current vapers (26.2%) did not know how or whether their nicotine strength had changed, and only 8.1% had increased their nicotine strength, including 1.1% of vapers who went from using no nicotine to using vaping liquids with nicotine.

Table 17. Change in nicotine strength since started to vape among current vapers, England 2021 (ASH-A, weighted data)

Change since started to vape	% (n)
Increased from no nicotine	1.1 (8)
Increased strength	7.0 (44)
Stayed the same	31.4 (221)
Decreased strength	34.0 (240)
Decreased to no nicotine	0.0 (0)
Always no nicotine	0.3 (2)
Don’t know*	26.2 (175)

Notes: Age 18+. Unweighted base = 690; Current vaping included people who had tried vaping and who still vaped, excluding those who no longer vaped.

* This option was not included in previous reports, so this table is not directly comparable across reports.

4.11 Urges to vape and smoke

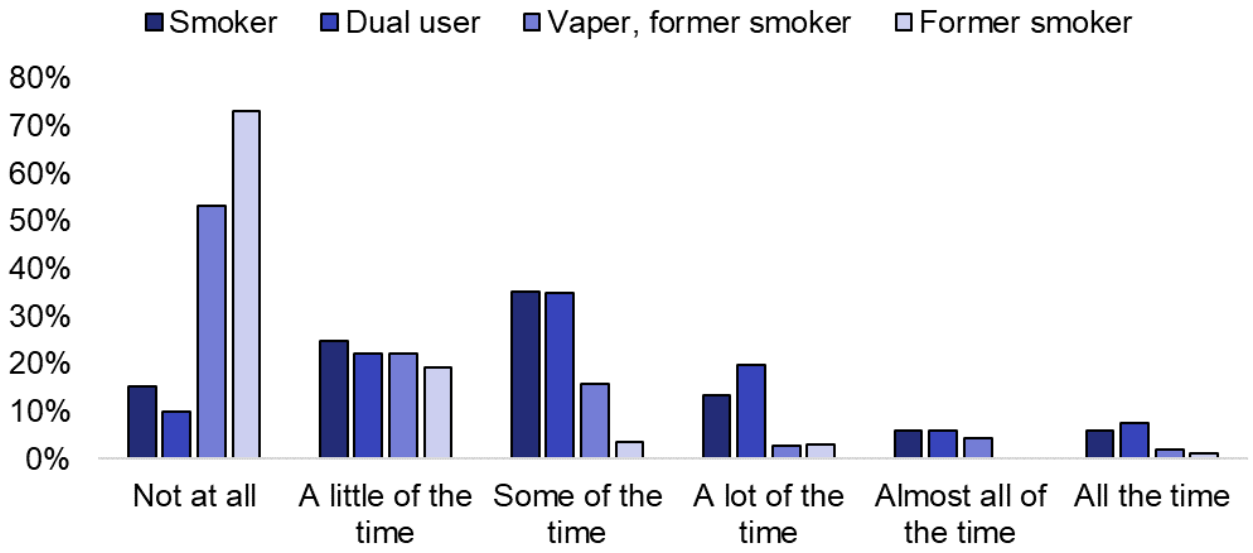
The STS survey assessed frequency and strength of urges to smoke (for former smokers, former smokers who were vaping, ‘dual users’ and smokers, figure 19), and ASH-A assessed the strength of urges to smoke (for ‘dual users’ and smokers) and vape (for vapers and ‘dual users’, figure 20).

Regarding urges to smoke (STS - figure 19), both groups of former smokers—non-vapers and those who were using vaping products—reported least frequent urges (92.4% and 75.4% experiencing urges to smoke ‘not at all’ or ‘a little of the time’ respectively) compared with ‘dual users’ (31.8%) and smokers (39.8%). In addition, strong, very strong or extremely strong urges to smoke were reported by 30.8% of ‘dual users’, 21.3% of smokers and 14.3% of former smokers who were using vaping products, suggesting stronger nicotine dependence among ‘dual users’.

In the 2021 ASH-A data, strength of urges to vape and/or smoke (ASH-A - figure 20) appear relatively similar across vapers, ‘dual users’ and smokers, with the largest proportions reporting moderate strength urges.

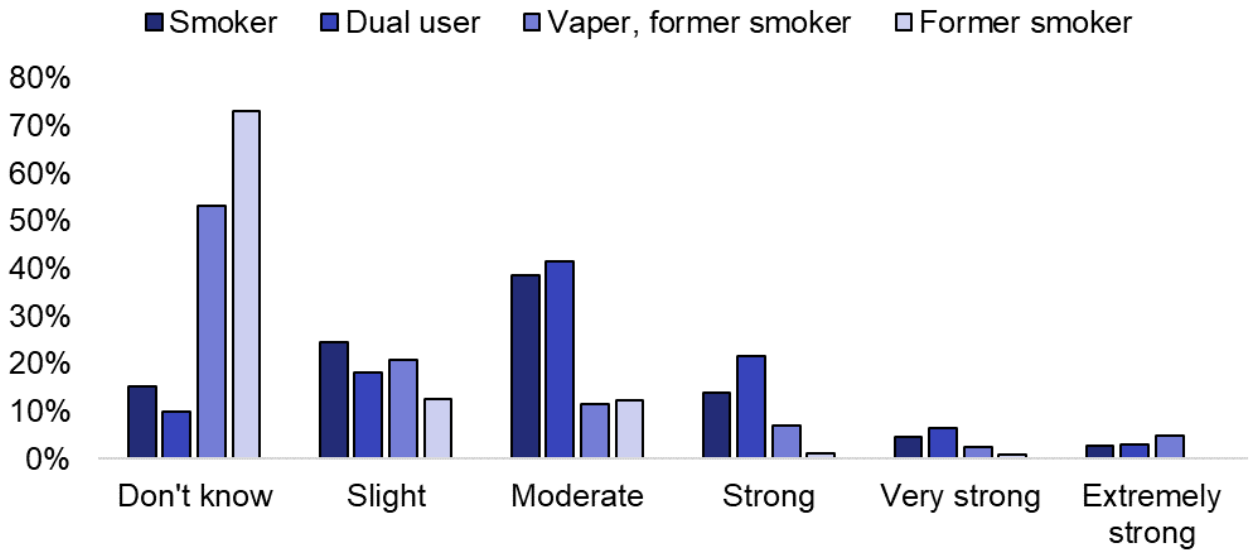
Figure 19. Frequency and strength of urges to smoke among smokers, dual users, vapers and former smokers who had stopped smoking in the last year, England 2021 (STS, weighted data)

Frequency of urges to smoke



Notes: Age 18+. Unweighted bases: Smokers=1,537; Dual users=427; Vapers=88; Former smokers=240.

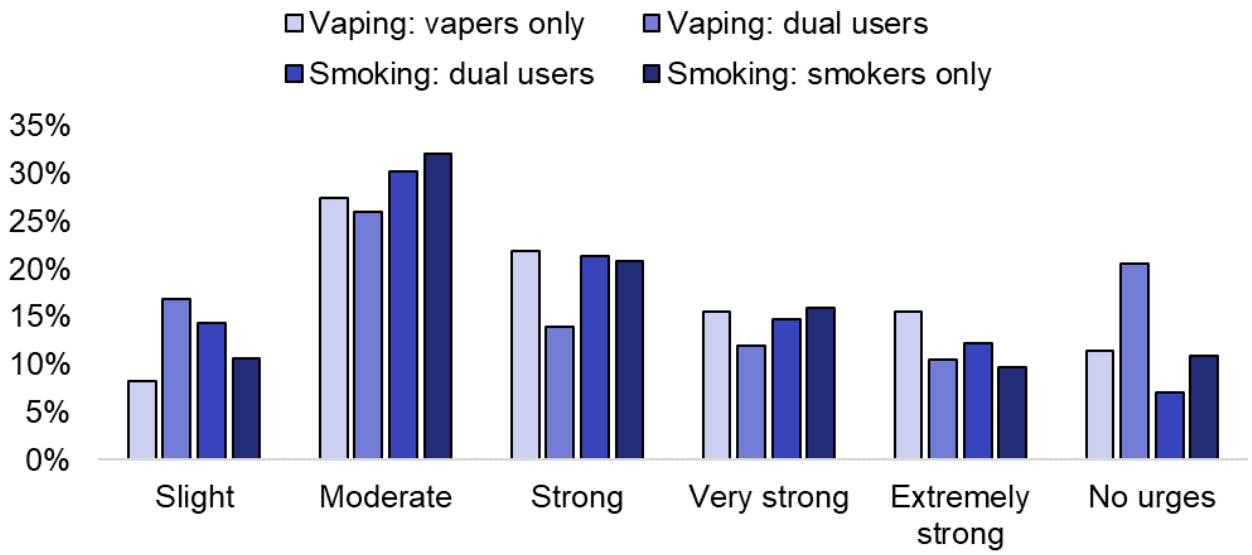
Strength of urges to smoke



Notes: Age 18+. Unweighted bases: Smokers=1,512; Dual users=424; Vapers=88; Former smokers=240.

Current smoker included people who said that they smoked daily or that they smoked, but less than daily, and were not currently vaping. Dual user included current smokers who also ‘currently vaped for any reason’. Current vaper included people who ‘currently vaped for any reason’ and stopped smoking in the last year. Former smoker included people who stopped smoking in the last year and are divided into whether they were or were not currently vaping. STS data available from January to September 2021.

Figure 20. Strength of urges to vape among current vapers and dual users and strength of urges to smoke among current smokers and dual users, England 2021 (ASH-A, weighted data)

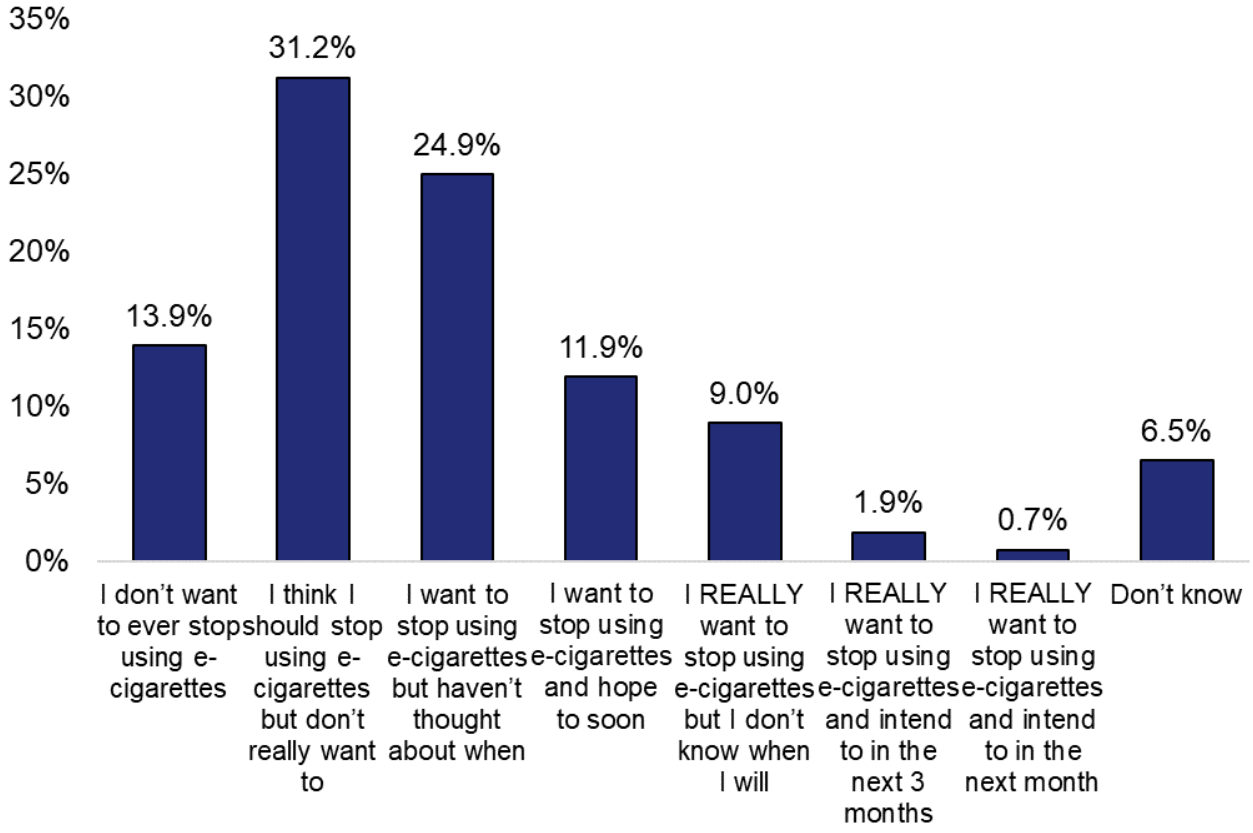


Notes: Age 18+. Unweighted bases: Vaping = 690; Smoking = 1,202. Current vapers included people who had tried vaping and who still vaped. Dual users included people who had tried vaping and who still vaped but also smoked daily or not daily. Current smoking included people who smoked daily as well as those who smoked, but not daily and excluded people who also vaped.

4.12 Motivation to stop vaping

The 2021 ASH-A survey asked former smokers who were currently using vaping products how much they wanted to stop vaping (figure 21). Responses were skewed towards lower motivation to stop vaping, with 70.0% of former smokers responding that they “don’t want to ever stop” (13.9%), “don’t really want to stop” (31.2%) or “want to stop but haven’t thought about when” (24.9%) to stop using vaping products. Only 2.6% of former smokers responded they were motivated to stop using vaping products in the next one or 3 months (figure 21).

Figure 21. Motivation to stop using e-cigarettes among former smokers who currently use vaping products, England 2021 (ASH-A, weighted data)



Notes: ASH-A (18+): Unweighted base = 446. Former smokers who currently use vaping products included people who had stopped smoking, had tried vaping and who still vaped.

4.13 Other nicotine products

Use of heated tobacco products (HTP) is described in a separate chapter (chapter 14). Briefly, HTP use has remained low at 0.3% among current or past year smokers in the STS and 0.5% among adults in the ASH-A survey in 2021 (0.2% and 0.3% respectively in 2020).

The 2021 ASH-A survey also reported that approximately 0.4% of adults in England had tried and still used nicotine pouches in 2021 (0.5% in 2020). In 2019 and 2020, ASH-A also asked about tobacco products that are chewed or sucked; in both years,1.2% reported using them once a week or more often. In general, use of HTP, chewed or sucked tobacco products or nicotine pouches show little to no evidence of increase over time.

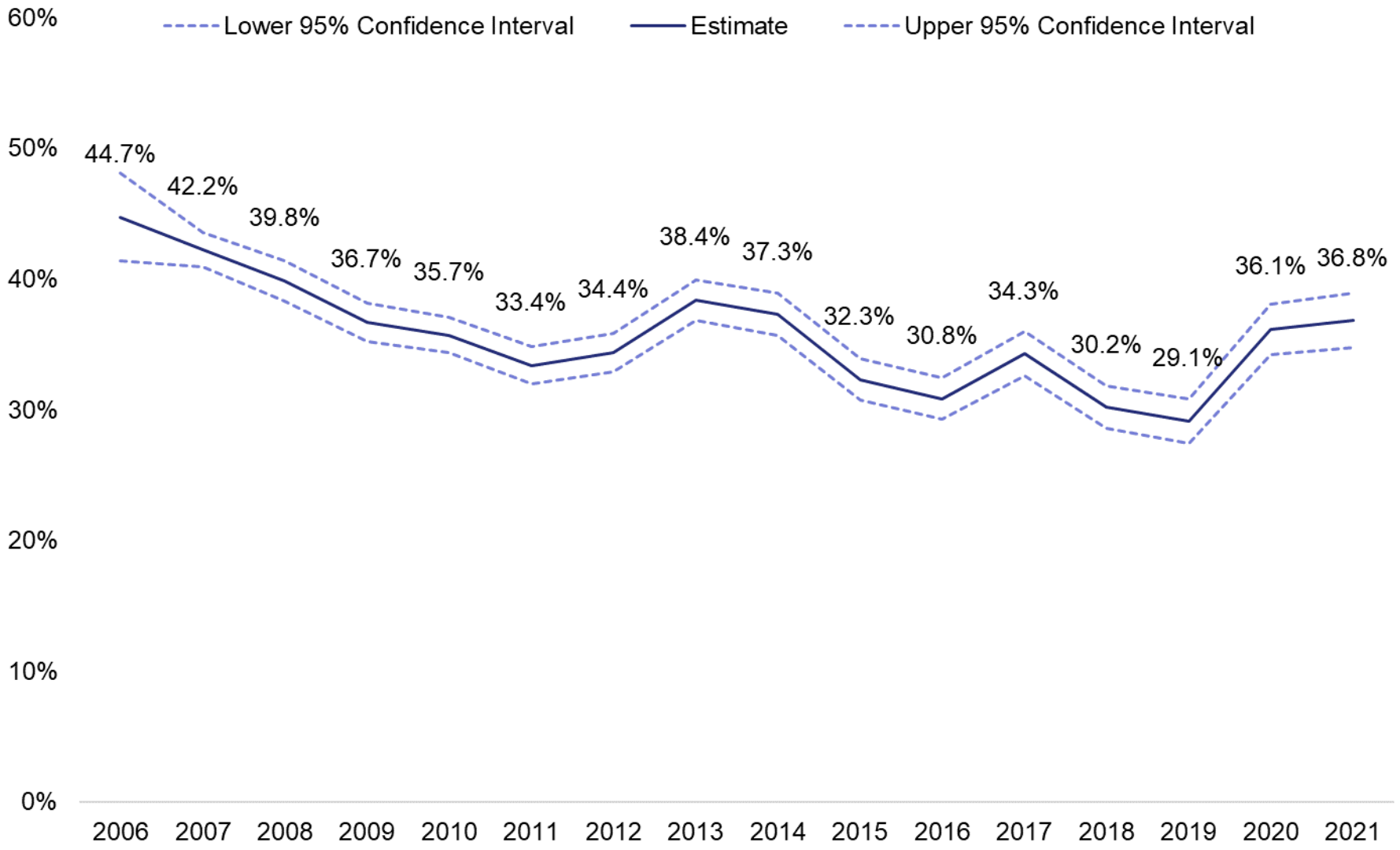
4.14 Vaping and smoking cessation in England

The following paragraphs give an overview of the use of using vaping products for smoking cessation in England. By using the STS data, we present the latest trends on stop smoking attempts, stop smoking success rate and how often vaping products and other smoking cessation aids are used by smokers when trying to quit smoking in England. In addition, we provide an update on the use of vaping products and associated smoking cessation outcomes in English stop smoking services.

Figure 22 and figure 23 illustrate the proportion of people between 2006 to 2007 and September 2021 who tried to quit and were successful, respectively. Those trying to quit are defined as past year smokers who made at least one serious attempt to stop smoking (that is, they decided that they 'would try to make sure they never smoked again') in the previous 12 months. Those who were successful are people who report still not smoking after a quit attempt made at any point within the past 12 months.

The proportion of smokers who reported trying to quit smoking has fluctuated since 2011, with the highest proportion in 2013 (38.4%) and the lowest in 2019 (29.1%, figure 22). Since 2019, this proportion has increased to 36.1% in 2020 and 36.8% for the January to September period in 2021 (figure 22). Alongside the increase in attempts to stop smoking, success rates for those who reported they had tried to stop smoking in the previous year have also increased—from 14.3% in 2019 to 21.6% in 2020 and 25.1% in 2021 (figure 23). These sharp upward trajectories in proportions of quit attempts and quit success may have been prompted by the COVID-19 pandemic. As described in Section 4.2, Jackson and others (5) analysed STS data collected before (August 2019 - February 2020) and after the first national lockdown (April - July 2020) and compared it with smoking and quitting smoking behaviour during the same time periods in 2018 to 2019. Their study concluded that the first lockdown in England was associated with a substantial increase in stop smoking attempts, particularly among 18 to 34 year olds, compared with a year before, and that the rate of smoking cessation increased by 156.4% in relation to quit attempts before the lockdown (August 2019- February 2020) and did not differ across smokers' age, gender or social grade (5).

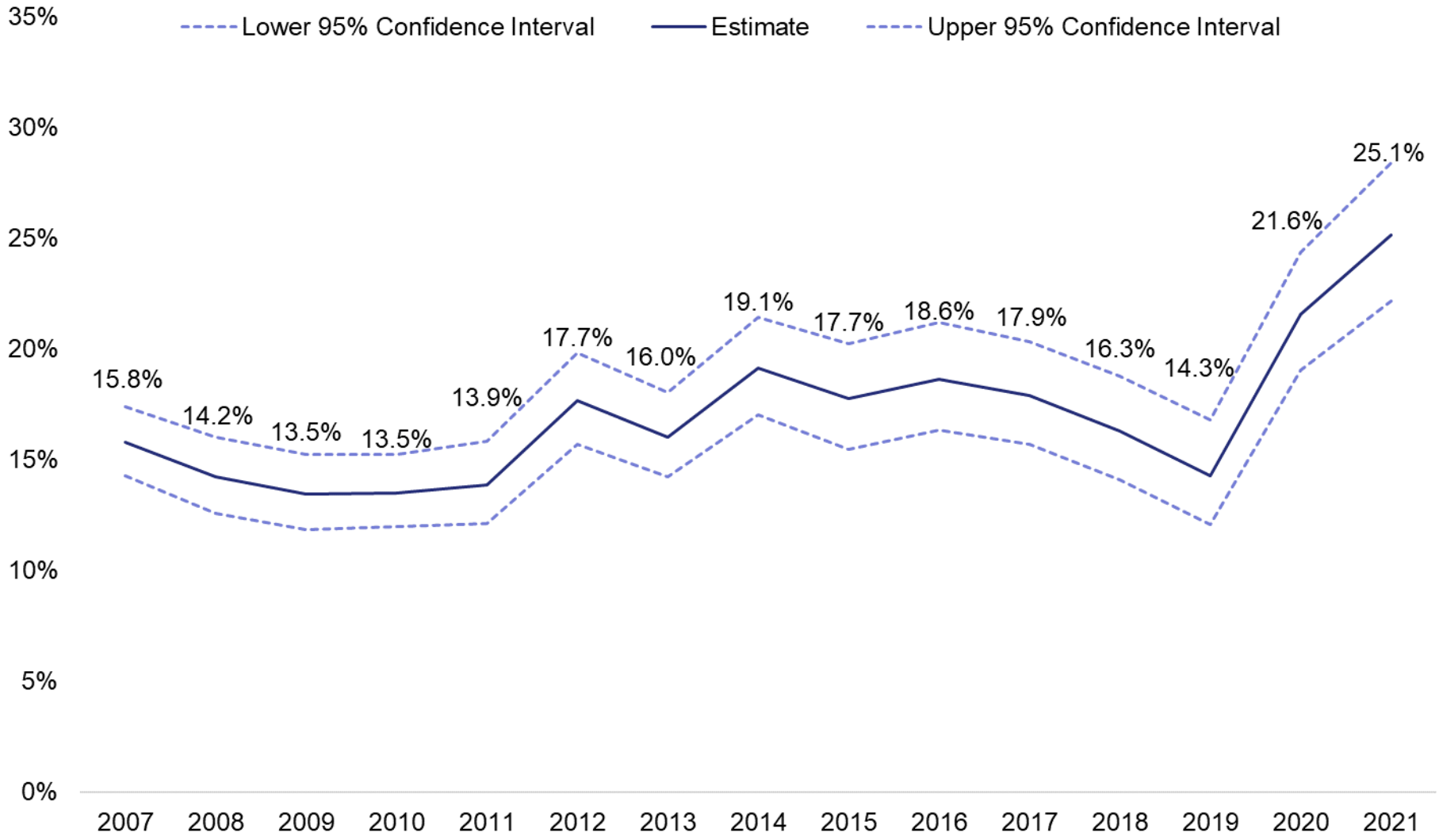
Figure 22. Proportion of people who tried to stop smoking in the past year, England 2006 to 2021 (STS, weighted data)



Notes: Unweighted bases 2007=6,114; 2008=4,735; 2009=5,069; 2010=5,995; 2011=5,190; 2012=5,063; 2013=4,549; 2014=4,021; 2015=4,047; 2016=3,831; 2017=3,515; 2018=3,630; 2019=3,308; 2020=2,915; 2021 (January to September)=2,466. Base: adults (age 18+) who smoked in the past year.

Percentages in the graph refer to the estimate.

Figure 23. Success rate for those who tried to stop smoking in the past year: England 2007 to 2021 (STS, weighted data)



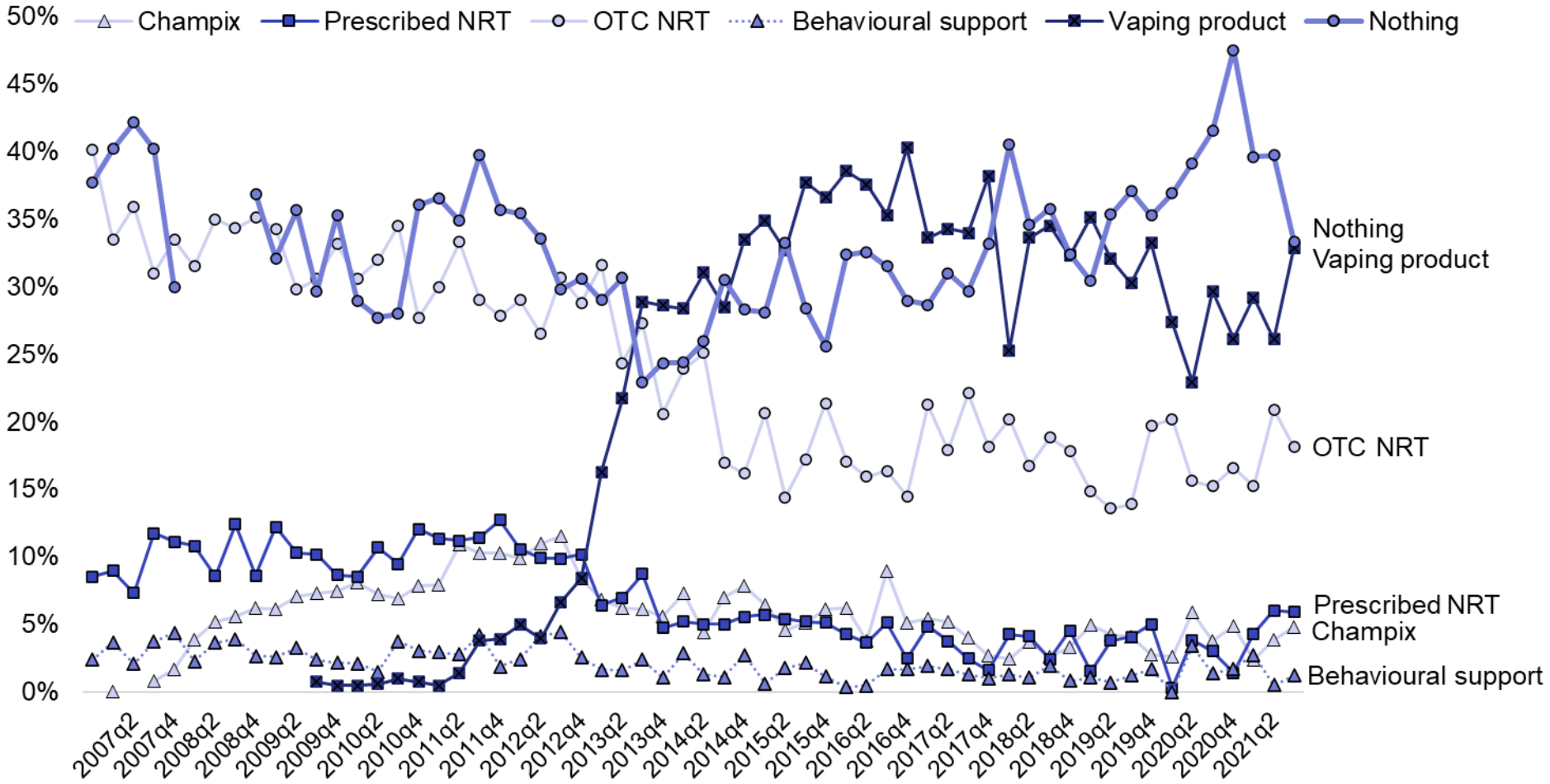
Notes: Unweighted bases: 2007=2,565; 2008=1,860; 2009=1,839; 2010=2,101; 2011=1,698; 2012=1,708; 2013=1,728; 2014=1,483; 2015=1,283; 2016=1,139; 2017=1,201; 2018=1,085; 2019=973; 2020=1,041; 2021 (January to September)=880. Base: adults (age 18+) who smoked in the past year and who tried to stop in the preceding 12 months.

Percentages in the graph refer to the estimate.

4.15 Use of vaping products for smoking cessation in England (population level data)

As described in our previous reports (2, 15-18), vaping products have remained the most popular aid used by STS participants in their most recent quit attempt—for the first 3 quarters of 2021, 29.5% of smokers attempting to stop reported having used a vaping product in their attempt (figure 24). Around 18% used NRT bought over the counter (OTC) and around 5% used NRT on prescription as an aid in their most recent stop smoking attempt. The smoking cessation medication varenicline (Champix), and behavioural smoking cessation support were used least often by smokers attempting to quit in the past year (figure 24).

Figure 24. Proportion of smokers trying to stop by support used in most recent quit attempt, England 2007 to 2021 (STS, weighted data)



Notes: n=17,191 for vaping products, 23,001 for all others. Adults aged 18+ who smoke and tried to stop or who stopped in the past year. OTC NRT = Nicotine replacement therapy over the counter; Prescribed NRT = Nicotine Replacement Therapy on prescription; Champix = Varenicline.

4.16 Use of vaping products in stop smoking services in England

Introduction

The COVID-19 pandemic has affected stop smoking services. Between August and September 2020, ASH conducted the seventh annual survey of tobacco leads in English local authorities with responsibility for public health (19), and its eighth annual report in August 2021 (20). These reports track the key indicators of the state of stop smoking services and wider tobacco control functions across local government settings. The most recent survey included responses from 118 out of 126 local authorities (84%). Three-quarters of surveyed local authorities (76%, n=85) reported they commissioned a specialist stop smoking service, although 13 local authorities restricted access to specific groups of the population, such as pregnant women or people with long-term health conditions. Overall, 67% of local authorities offered a universal specialist service, up from 62% in 2020.

The COVID-19 pandemic forced local authorities to reconfigure their stop smoking services. In 2020, almost all services stopped offering face to face support and instead offered telephone support (98%). At the time of the survey in August 2021, this change had been largely reversed and face-to-face advice was on offer in 83% of surveyed local authorities. Video conferencing has been the greatest innovation within services. Prior to the pandemic, only 29% of local authorities had commissioned this method, but by the time of the surveys in 2012 and 2021, 60% were using it. Some negative effects of these remote behavioural support methods were reported, including regret at the loss of face-to-face advice, the risk of excluding some clients without access to technology or specific needs and the loss of CO monitoring. However, these remote behavioural support methods were reported to be widely welcomed by clients as they were more flexible and accessible than face-to-face appointments.

In the past couple of years, stop smoking services found new ways of ensuring that their clients could obtain NRT, other medications and vaping products. These included emailing vouchers and letters to pharmacists and GPs, by using online pharmacies and delivering them directly to clients' homes. There has been a small improvement in the medications offered over the last 2 years. In 2021, 76% of surveyed local authorities offered a full course of dual NRT, compared to 65% pre-pandemic. Overall, 88% of surveyed local authorities offered smokers a full 12-week course of varenicline (Champix) in 2021, though in practice Champix has not been available to prescribe since late 2021 (see chapter 1). The biggest improvement however has been seen with the provision of vaping products as part of the support offered by stop smoking services. In 2019, only 11% of surveyed local authorities offered vaping products to some or all smokers accessing stop smoking

services. In 2021, 40% of surveyed local authorities offered vaping products to some or all smokers and a further (15%) had plans to do so (20).

In November 2021, National Institute for Health and Care Excellence (NICE) issued new guidance on tobacco uptake and cessation (21). This included updated advice on the use of nicotine-containing e-cigarettes (see chapter 1).

English stop smoking services - delivery and outcome measures

Stop smoking services offer behavioural support in addition to licensed medication (NRT, varenicline, bupropion). Vaping products, alone or in combination with licensed medication, concurrently or consecutively are also used. A small number of stop smoking services offer vaping products as part of the provision of support. In other services, where vaping devices are not directly provided, some people making a quit attempt use their own vaping product while receiving behavioural support (alone or alongside licensed medication).

Data are collected by NHS Digital from local authority commissioned services every 3 months about: the number of quit attempts made (people can make several quit attempts in one year and therefore be counted more than once); the number of quit attempts which led to successful quits at 4 weeks (self-reported and carbon-monoxide (CO) verified); and key measures of the service including intervention type, intervention setting and type of pharmacotherapy received. A person is counted as a 'self-reported four-week quitter' if they are assessed (face to face or by telephone) 4 weeks after the designated quit date and declare that they have not smoked a single puff on a cigarette in the past 2 weeks. A person is counted as a CO-verified 4-week quitter if they are a self-reported 4-week quitter and their expired-air CO is assessed 4 weeks after their designated quit date (-3 or +14 days) and found to be less than 10 parts per million. People who have set a quit date and are lost to follow up are counted as non-quitters.

Although some stop smoking services are commissioned to provide extended behavioural support beyond 4 weeks post-quit date, NHS Digital only requires the submission of data regarding quit attempt outcomes after 4 weeks. Four-week CO verified quit rates represent a reliable and valid indicator of smoking cessation which can be used to predict long-term abstinence rates and provide a good balance between accuracy and practicability (22). In 2020 to 2021, CO monitoring was generally not possible due to COVID-19 related restrictions. The comparative quit rates with different types of support have also been found to be stable over longer follow ups (23).

The number of quit attempts made with stop smoking services and the number of self-reported quitters have declined annually since a high of 816,444 in 2011 to 2012. Between April 2020 and March 2021, 178,815 quit dates were set with a stop smoking service, 57.6% set by women and 42.4% by men. The majority (85.7%) of those setting a quit date

were of white ethnicity, 5.1% did not state their ethnicity, 3.9% were of Asian or Asian British, 2.0% mixed, 1.8% Black or Black British and 1.4% of other ethnic groups. At 4-week follow up, 105,403 (58.9%) self-reported that they had successfully quit, with 61.7% of men and 56.9% women reporting success. The self-reported quit rate varied, from 54.8% for those without stated ethnicity to 59.3% for people of white and 59.3% for people of Asian or Asian British heritage (24). The largest number of quit attempts were made by people who were classified as being from 'routine and manual occupational groups' (48,623, 28.4%) of whom 61.6% successfully quit (compared with 63.7% among those from 'managerial/professional occupational groups').

Use of vaping products in quit attempts supported by stop smoking services

NHS Digital provide numbers and proportions of quit attempts and quit success by each type of pharmacotherapy (including vaping products) used in the quit attempt. However, they do not provide additional information that may influence quit success (for example level of tobacco dependence, age, socio-economic status according to type of pharmacotherapy offered). Therefore, these data only allow for a crude comparison between and within stop smoking services. This report presents the most recent data (at the time of writing the report) provided by NHS Digital from 1 April 2020 to 31 March 2021. Previous reports provide information about use of vaping products in quit attempts from April 2014 onwards (2, 15-18).

Combination NRT remained the most frequently used type of pharmacotherapy in a quit attempt (33.5%) and varenicline was the second most frequent cessation aid (28.8%, table 18). A vaping product was used in 5.2% of quit attempts either alone (2.2%), concurrently (2.2%) or consecutively in combination with licensed medication (0.8%). The highest quit rates were observed when the quit attempt involved the use of a licensed medicine (that is, prescription NRT, bupropion or varenicline) and a vaping product concurrently (67.8%), followed closely by those using a vaping product and licensed medication consecutively, or varenicline alone (both 66.7%); those using a vaping product alone achieved a 4-week quit rate of 61.5% (table 18).

Table 18. Type of stop smoking support, associated success rates and proportion of quit attempts using this support, England April 2020 to March 2021 (NHS digital, n=178,815)

Type of stop smoking support	Self-reported 4-week success rate, %	Quit attempts using the type of support, %
Licensed medication and vaping product concurrently	67.8	2.2
Varenicline only	66.7	28.8
Licensed medication and vaping product consecutively	66.7	0.8
Vaping product only	61.5	2.2
Bupropion only	59.8	0.3
Single NRT	59.3	19.8
Combination NRT	55.0	33.5
None	52.6	8.0
Licensed medications consecutively	47.5	1.8
Unknown	36.2	2.6

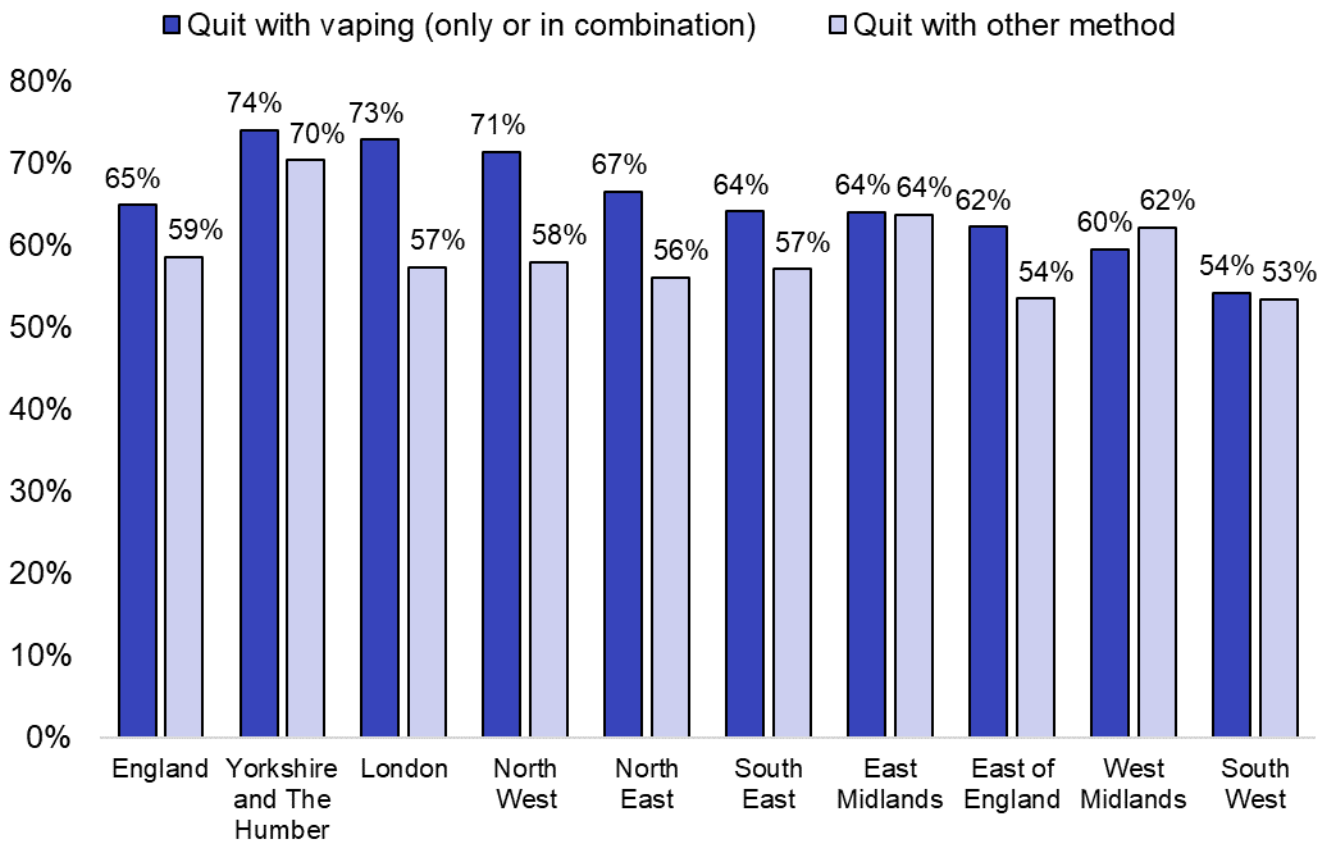
Vaping in quit attempts in stop smoking services by region

Vaping as part of a stop smoking service supported quit attempt continues to vary by region, from 2.3% in London to 11.9% in the East Midlands (table 19). Across the regions, quit rates in supported quit attempts with a vaping product ranged from 54.2% in the South West to 74.1% in Yorkshire and the Humber and in many regions appeared higher than quit attempts using other methods (figure 25).

Table 19. Proportion of quit attempts involving a vaping product in England overall and by region (NHS Digital)

Region	%
England	5.2
East Midlands	11.9
South East	7.6
East of England	6.2
North East	4.5
South West	4.4
West Midlands	3.7
North West	3.3
Yorkshire and the Humber	3.2
London	2.3

Figure 25. Self-reported 4-week successful quits using a vaping product compared with other methods in England and by region (April 2020 to March 2021, NHS Digital)



Notes: Other methods include single NRT, combination NRT, varenicline, bupropion, behavioural support only and pharmacotherapy unknown.

Limitations of the data

It is possible that people using a vaping product for stopping smoking, alone or in combination with other licensed stop smoking medicines, may differ in their demographic, clinical or smoking characteristics from people making a quit attempt with licensed medication only or behavioural support only. People who attend stop smoking services are self-selected and, since 2014, the reporting of activity by commissioned stop smoking services to NHS Digital has been voluntary; for 2020 to 2021, 14 local authorities (9.3% out of 151) did not submit data. Although most services continue to report to NHS Digital, it is possible that those who do not may be more (or less) effective in supporting smokers to quit with the use of vaping products.

Notwithstanding the limitations of the data, people who are treated by a stop smoking service with behavioural support and use a vaping product with or without additional licensed medication, have at least comparable 4-week quit success rate compared with

people who used licensed medication only. However, the proportion who use vaping products as part of a supported quit attempt remains very small. This contrasts with findings from the STS (figure 24) that suggest vaping products remain the most popular stop smoking aid in people who reported making a quit attempt in the previous year. The most likely reasons for this difference are that many people who use a vaping product to help them stop smoking do not actively seek support from stop smoking services and few services offer vaping products. As suggested in our previous reports, stop smoking services may not be actively reaching out or have the resources to support people who may want to use a vaping product as part of a quit attempt.

4.17 Conclusions

Data reported in this chapter came from 4 different surveys. Most data were from the STS, collected between January and September 2021, and the 2021 ASH-Adult (ASH-A) survey, collected in February and March 2021. Other data from the OPN and APS surveys were collected in 2020. We also report some data from the most recent 2022 ASH-A survey on smoking prevalence, vaping prevalence, the relationship between smoking and vaping and type of vaping products used.

Smoking prevalence among adults in England in 2021 was between 12.7% and 14.9% depending on the survey and in 2022, based on ASH-A data, 13.2%. These equate to about 5.6 to 6.6 million smokers.

There was variation in smoking prevalence by age, gender, socio-economic status and ethnicity. Most notably, smoking prevalence remained significantly higher among adults from more disadvantaged groups.

Vaping prevalence among adults in England was lower than smoking prevalence across all groups and seemed to have increased by around 1 percentage point from 2020 to 2021, to between 6.9% and 7.1%, equating to about 3.1 to 3.2 million vapers. In 2022, based on ASH-A data, adult vaping prevalence in England was 8.3%.

There was some variation in vaping prevalence by socio-demographic groups and smoking status. Using 2021 STS data, the highest vaping prevalence was among men (7.8%), people from the north of England (8.3%), people from social grades C2, D and E (8.8%) and among current smokers (22.0% compared with 11.6% among former smokers and 0.6% among never smokers). Among former smokers, 27.9% of short-term former smokers (quit for less than one year) used vaping products, compared with 9.9% of long-term former smokers (quit for longer than one year). This is an increase since 2013 when 1.2% of long-term former smokers vaped. In comparison, a small but steady proportion of long-term former smokers have used NRT (around 2% to 4%) since 2013.

The proportion of vapers who also smoke had been declining since 2012, from 91.9% to 49.8% in 2020 in the STS survey and from 73.7% to 31.0% in 2021 in the ASH-A survey. However, both STS and ASH-A surveys suggest a recent increase in the proportion of vapers who smoke. The STS survey showed an increase to 51.7% in 2021, and the ASH-A survey showed an increase to 33.4% in 2022. The discrepancy in estimates across surveys is likely due to different definitions of smoking status.

In both STS and ASH-A, tank models remained the most popular type of vaping device, used by 59.3% of current vapers in the STS 2021 survey and 64.3% of current vapers in the ASH-A 2022 survey. Modular vaping products were used by 20.1% of current vapers, cartridge models by 14.9% and disposables by 4.6% in the STS 2021 survey. The 2022 ASH-A survey showed higher use of disposable vaping products than in 2021, with 15.2% of current vapers reporting using disposable vaping products in 2022 compared with 2.2% in 2021.

Among adults who had ever vaped, daily vaping was associated with their smoking status. Among never smokers who had ever vaped, nearly two-thirds (64.9%) had tried it once or twice and 5.0% were vaping daily. Among current daily or non-daily smokers who had ever vaped, around 27% vaped daily. Among former smokers who had ever vaped, more than half (57.7%) vaped daily (2021 ASH-A.

2021 ASH-A data suggested an increase in the proportion of current vapers who have vaped for more than 3 years (23.7% in 2018, 29.3% in 2019, 39.2% in 2020 and 43.7% in 2021). People who had vaped in the past mostly stopped after 6 months of use or less (57.2% in 2021).

The most common reasons for vaping reported in the 2021 ASH-A survey were to quit (27.9%) or stay off (17.7%) smoking tobacco or because people enjoyed it (12.6%).

In 2021, strengths of vaping liquids above those allowed by regulations (more than 20mg/mL) were used by less than 6% of vapers. Just over a third of vapers (34.0%) reported reducing the strength of the nicotine vaping liquid they use since starting to vape, 31.4% continued using the same strength and 26.2% did not know if they had changed the strength. Just 8.1% of people reported having increased the strength of the nicotine in vaping liquid they use since starting to vape (2021 ASH-A). The proportion of vapers unsure about the strength they are using has increased slightly over the last 2 years.

Fruit (35.3%), menthol/mint (22.5%) and tobacco (20.9%) remained the most popular flavours among vapers (2021 ASH-A).

Attempts to stop smoking and success rates for those who tried to stop smoking increased significantly in the last 2 years, most likely due to the COVID-19 pandemic. Vaping products remained the most common aid used in a quit attempt.

Stop smoking services have greatly improved the provision of vaping products to support a quit attempt. In 2019, 11% of surveyed local authorities offered vaping products to some or all smokers accessing stop smoking services. In 2021, 40% of surveyed local authorities offered vaping products to some or all smokers and a further 15% had plans to do so.

Between April 2020 and March 2021, quit attempts in stop smoking services that involved the use of a vaping product (alone or in combination with medication) achieved self-reported short-term success rates of 64.9%, compared with 58.6% for attempts not involving a vaping product. Despite this, only 5.2% of supported quit attempts involved a vaping product.

4.18 Implications

Vaping is more common among disadvantaged adult groups in society. This mirrors smoking prevalence, and research should continue to explore the impact that higher vaping prevalence has on stopping smoking and reducing health inequalities.

The continuing impact of COVID-19 on smoking and vaping among adults needs to be monitored. This should include younger adults who start smoking and vaping and any changing patterns in the data.

There needs to be further research into the increasing proportion of long-term vapers and their motivation to stop vaping, and whether people who want to stop vaping need support. More research is also needed into vaping among never smokers, younger adults and people from ethnic minority backgrounds.

A recent increase among these groups of using disposable vaping products warrants further monitoring and research.

Recently issued NICE guidance ‘Tobacco: preventing uptake, promoting quitting and treating dependence’ should encourage more stop smoking services to support smokers who want to stop smoking with the help of a vaping product.

As we recommended in previous reports in this series, and supported by the new NICE guidance, all smokers should be supported to stop smoking completely, including dual users who smoke and vape.

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5 Nicotine

5.1 Introduction

Objective

This chapter begins with a brief overview of the role of nicotine in smoking and vaping product use and the 2 main concerns in relation to nicotine, nicotine dependency and toxicity. The objective of this chapter is then to summarise:

- data on the use of nicotine in vaping products among youth and adults in England
- evidence from previous reports on nicotine dependency
- evidence from previous reports on nicotine toxicity
- updated evidence from the systematic reviews covered in this report

Overview of role of nicotine in smoking and vaping product use

Since the 1970s, nicotine has been recognised as the primary reason why people smoke (1, 2), but not the major contributor to the overall harmfulness of smoking (3 to 5). Alternative less harmful nicotine delivery systems were therefore developed initially for therapeutic reasons: as short-term aids to smoking cessation to reduce nicotine withdrawal symptoms while smokers dealt with the absence of behavioural and psychological aspects of smoking (6). Subsequently however, the prospect of recreational cleaner nicotine delivery devices was mooted as a potential solution to the tobacco epidemic (7). The principle was to purify the drug and its delivery system as much as possible and provide an ‘acceptable source of purer, less contaminated nicotine’. Russell (1991) proposed that ‘nicotine use should not be presented as something good, but rather as something far less bad than tobacco’ (7). He argued that some palatable and acceptable nicotine replacement products be actively promoted on the open market so that they could compete with tobacco products.

Thirty years on however, there is no consensus on recreational nicotine use. There are 2 pertinent issues. First, nicotine dependency is controversial. In contrast to Russell’s perspective, some argue that nicotine dependency is a recognised disorder and the compulsive use interferes with daily life. While nicotine dependency does not impair everyday functioning in the same way as other drugs, and may enhance mood and functioning directly as well as through withdrawal relief, it is correlated with propensity to use (and to some extent the attractiveness of tobacco products), and so is the main problem that needs to be controlled. These considerations are further complicated by the

array of factors other than nicotine contributing to dependency. The added complexity is that a nicotine product that is less harmful than smoking tobacco and which has high dependency potential, may facilitate smoking cessation, but could also facilitate young people who have never smoked to initiate vaping and potentially maintain use over a prolonged period. These issues are also explored elsewhere, for example, in published research (8, 9). The second main issue is the extent to which there are health risks associated with nicotine (and relatedly, health risks associated with the nicotine replacement products themselves). Both dependency and health issues are relevant to the positioning of vaping products and are explored further below.

In relation to terminology in this section, a key uncertainty is how best to define and assess ‘dependency’. Several terms are used to describe dependency of tobacco and nicotine products and often these are used interchangeably (2, 10). The terms dependency, addiction, disorder and withdrawal have largely been driven by the World Health Organization International Classification of Diseases (ICD-10) and the American Psychiatric Association’s Diagnostic and Statistical Manual (DSM-V) criteria. An additional term used is abuse liability or abuse potential, defined by the US Food and Drug Administration as "the likelihood that abuse will occur with a particular drug product or substance with CNS (central nervous system) activity". Drug abuse is defined as ‘the intentional, non-therapeutic use of a drug product or substance, even once, to achieve a desired psychological or physiological effect’ (11). The higher the abuse liability or potential, the more effective the vaping product may be in relation to smoking cessation. Overall in this chapter we prefer to use the term dependency to capture the propensity of a vaping product to induce continued use.

A key development in vaping technologies in recent years has been the introduction of products that use nicotine salts. Nicotine is a chemical with a weak base and absorption is pH dependent, with lower absorption in lower pH (more acidic) environments. Nicotine can either be freebase or protonated (or bound), with protonation being higher in acidic environments where it less rapidly crosses membranes. Nicotine salts are formed when an organic acid is added to nicotine in e-liquids, resulting in salt formation. This reduces the harshness of the nicotine delivery so higher nicotine levels can be delivered. The most common protonating agents are carboxylic acids (for example, benzoic acid and levulinic acid). Some have said that this development mirrors the addition of acids to tobacco cigarettes in the 19th century through the use of flue-cured tobacco which resulted in protonated nicotine (pH 5.5 to 6) with little buccal absorption and quicker absorption in the lungs (12). Air-cured tobacco, mostly used in pipes and cigars has a higher pH (6.5 or above), a higher proportion of freebase nicotine and thus greater absorption in the mouth which is slower than through the pulmonary route but potentially has greater sensory effects (13). Other vaping product constituents, such as flavours, may also alter the pH and therefore nicotine absorption from vaping product aerosols.

Use of nicotine in vaping products and behaviours

The use of nicotine in vaping products was discussed in chapters 3 (Vaping among young people) and 4 (Vaping among adults). We summarise the relevant findings here augmented where possible with comparisons with other countries.

Data from the Smoking Toolkit Survey (STS) indicated that in 2021, a minority of adults (13.2%) who were current vapers used non-nicotine e-liquids. Since 2016, this proportion had varied little between 11.4% (2020) and 13.9% (2018). There are sparse comparable international data. One study, using data from a cohort of current vapers (daily, weekly or monthly) included in the International Tobacco Control Policy Evaluation Study (ITC) 2016 and 2018 adult surveys, indicated that in 2018, 6.0% of vapers in England reported no nicotine content in their vaping products, which was significantly lower than the proportion in Canada (12.9%), the US (8.6%) and Australia (11.4%) (14). Further exploration of these adult vapers is merited, for example in relation to duration of use of nicotine-free vaping products, and frequency of use. Overall, however, these data suggest that, similar to cigarette smoking, nicotine plays a central role in vaping.

As discussed in chapter 1 (introduction), the nicotine content of e-liquids is limited to 20 milligrams per millilitre (mg/mL). Since 2016, according to STS the most popular strength of e-liquid has been 6mg/mL or less, used by just over a third of current adult vapers, with the next most common strength being 12 to 19mg/mL used by around a quarter of vapers. The proportion who did not know the nicotine strength of their vaping liquids appears to have increased over the last few years, from 2.7% in 2019 to 9.1% in 2021. This is broadly consistent with cross-sectional data from the 2018 and 2020 waves of the International Tobacco Control (ITC) study. The ITC data indicated that in both 2018 and 2020, 6.7% of current daily or weekly vapers reported not knowing the nicotine strength of their e-liquids (compared with 15.4% and 5.2% in Canada, 9.8% and 9.7% in the US, and 5.7% and 13.6% in Australia in 2018 and 2020 respectively) (15). If the uncertainty in vapers' knowledge about their nicotine strength is increasing, it will be important to ascertain why.

ASH-A data from 2021 suggested most current vapers had either reduced (34.0%) or continued using the same (31.4%) strength of nicotine e-liquid since they began vaping. In the ITC cohort study referred to above (14), 89.3% of current vapers in England reported using the same nicotine content in 2016 and 2018, with 2.1% increasing and 8.7% decreasing; this compared with 85.9%, 6.1% and 7.9% in Canada, 79.4%, 6.4% and 14.2% in the US, and 82.4%, 0% and 17.6% in Australia using the same nicotine content, increasing and decreasing respectively. Overall, these data suggest that users have a preferred nicotine content of e-liquid they use over time, with some decreasing but only a very small minority increasing. This implies self-titration to a preferred nicotine intake.

In relation to nicotine salt technology, for adults, neither STS nor ASH-A ask specifically about the use of nicotine salts. However, in a study carried out in Great Britain in 2019 (16), most daily/non-daily vapers (73.0%) were either not aware of nicotine salts or did not

know if they were using them. Among vapers who were aware, 47.8% reported currently using nicotine salts when vaping. This contrasts with ITC data from 2020 which indicated that when asked about whether they had ever used the salt form of nicotine e-liquid, only 7.4% of daily or weekly vapers in England said they did not know, compared with 4.6% in Canada, 4.4% in the US and 4.0% in Australia (15). However, the proportion of salt forms of nicotine vaping products on the UK and international markets was rapidly increasing during this time period.

Among youth, ASH-Y data indicated that in 2021, around a fifth (20.4%) of current and former vapers aged 11 to 18 years said their vaping products never contained nicotine; around a third (34.2%) said they used vaping products that always contained nicotine, and 34.5% reported their products sometimes contained nicotine with 9.9% saying they did not know. From the ITC Youth 2021 data, among 16 to 19 year olds who had vaped in the past 30 days and had ever used vaping products with nicotine, 12.2% said their current product did not contain nicotine, 68.9% said their current products contained nicotine, 14.4% said some of their products contained nicotine, and the remaining 4.5% said they did not know.

The ITC Youth 2021 data also indicated that the majority (53.7%) of those who had vaped in the past 30 days and were currently using vaping products with nicotine, used e-liquids which were less than 20mg/mL (levels below this were not differentiated). In the ITC Youth 2021 data, there were fewer “don’t know” responses to the awareness of the inclusion of nicotine question, compared to the 2019 data reported in our prior report (17). Further analyses have indicated that the majority of those using the higher nicotine concentration vaping products reported using tank products (as the type of vaping product used most often in the past 30 days) and mostly commonly bought them online.

In relation to nicotine salt e-liquids, the ITC Youth 2021 survey indicated that just over half (53.1%) of those who had vaped in the past 30 days, who also currently vaped nicotine-containing e-liquids and were aware of nicotine salts, currently used nicotine salts. In the ITC Youth 2021 data, there were also fewer “don’t know” responses to the awareness of the inclusion of nicotine salts question, compared to the 2019 data reported in our last evidence review (17). In 2019, comparative ITC data indicated that past 30-day youth vapers in England who currently vaped nicotine containing e-liquids and were aware of nicotine salts, were less likely to report using nicotine salts (12.3%), compared with those in Canada (27.1%, AOR=2.77, 95% CI:1.93-3.99) and the US (21.9%, AOR=2.00, 95% CI:1.36-2.95) (18).

In summary, these data indicate a centrality of nicotine in adult vaping product use but perhaps not as predominant as nicotine is for adult tobacco smokers. This may reflect the greater heterogeneity of vaping products and/or potential interactions with other components including flavours, factors discussed in chapter 6 on flavours. Current data also suggest that among younger vapers, nicotine may not be a strong driver of use but

that might change among older vapers who are more experienced vapers, although the questions in the surveys varied and were asked of different user groups. Nicotine salts are used among youth and adults but given poor awareness and a lack of comparability in survey questions, accurate usage figures and direct comparisons between adults and youth in England cannot be made at this time.

5.2 Evidence from previous reports on nicotine dependency

When summarising recent reports, we use the terminology for dependency described in those reports. We include evidence on nicotine delivery and exposure which is clearly apposite to a discussion of nicotine dependency.

Summary of our previous evidence reports

Our 2015 evidence report (19) reviewed nicotine delivery and showed that the duration and frequency of puffs and mechanical characteristics played a major role in determining nicotine content in vaping product aerosols; across the middle range of nicotine levels, in machine tests using a standard puffing regime, nicotine content of e-liquids was weakly related to nicotine content in vaping product aerosol. The evidence at that time indicated that the use of a ‘cigalike’ vaping product could increase blood nicotine levels by around 5 nanograms per millilitre (ng/mL) within 5 minutes of use comparable to delivery from oral nicotine replacement therapy (NRT). However, experienced vapers using a tank vaping product achieved much higher blood nicotine levels over a longer duration, similar to those associated with smoking. The speed of nicotine absorption was generally slower than from cigarettes but faster than from NRT (19).

We reviewed nicotine dependency from different delivery devices in our 2018 report (20). We indicated that dose and rate at which nicotine reaches the brain influences its addictive potential. The puff-by-puff high-nicotine bolus delivery of nicotine through cigarette smoking, reaching the brain within 15 to 20 seconds of inhalation (faster than by intravenous injection), makes the cigarette the delivery device with the highest dependency potential. NRTs are less likely to induce dependency, as they deliver nicotine more slowly and at lower doses. Even faster acting NRT (for example, nasal and mouth nicotine sprays) which deliver peak nicotine levels within about 10 minutes (still considerably slower than cigarette smoking) are less likely to induce dependency; around 10% of nasal spray users maintain use for over a year with lower proportions for other products. We also compared dependency in smokers with smokeless tobacco users, in particular snus, for whom overall nicotine exposure can be comparable. Nicotine absorption in snus users is through the buccal route, thus having a slower absorption rate than cigarette smoking. This indicated that factors other than speed of delivery can influence dependence, such as the inclusion of tobacco in products and pH levels (20).

In our 2018 report (20), we appraised nicotine delivery of vaping products as one assessment of the potential for nicotine dependency from vaping products. We indicated how nicotine delivery varied considerably across different vaping products, and that with the same puffing regime, experienced users could achieve greater increases in blood nicotine levels than naïve users. Experienced users could achieve at least comparable venous blood nicotine levels to cigarette smokers. Nicotine levels in experienced users of later generation devices peaked within 2 to 5 minutes after puffing a vaping product, indicating pulmonary delivery and likely dependency. Ad libitum vaping patterns differed from smoking patterns as vapers took longer puffs and grouped puffs together in shorter clusters or sessions (2 to 5 puffs); this led to more gradual rises in plasma nicotine levels in contrast to the bolus dosing from cigarette smoking described earlier. Nicotine intake was related to puff topography but only for tank users.

In summary, our previous reports concluded that as vaping products evolved, their nicotine delivery had improved. As alluded to earlier, we reported that this could mean that their dependency potential increased but that this could also make vaping products more attractive to smokers as a replacement for smoking. It was not clear at that time how vaping products with the fastest nicotine delivery characteristics compared with nicotine delivery of tobacco cigarettes.

The National Academy of Science, Engineering and Medicine (NASEM, 2018)

In its chapter entitled ‘Nicotine’, the NASEM report (21) drew on extant authoritative reviews on nicotine. Similar to our prior evidence reviews, NASEM indicated that the ‘abuse liability of tobacco products increases with greater delivery, faster rate of absorption and higher blood nicotine concentrations’ and that smoking was ‘the most reinforcing and dependence-producing form of nicotine administration’. NASEM indicated that vaping products delivered nicotine through the pulmonary route, similar to that in tobacco cigarettes.

In relation to abuse liability of vaping and the extent to which vaping products could help tobacco smokers stop was examined by NASEM through addressing the question ‘What is the nicotine exposure profile of e-cigarettes?’ and reviewing evidence on the amount of nicotine delivered and how it is delivered. They included 27 clinical studies that measured biomarkers of nicotine exposure including pharmacokinetic parameters such as maximum blood nicotine concentration (C_{max}), and time to maximum concentration (T_{max}). Additionally, they examined 7 studies in which smokers switched to vaping products for a study period and assessed biomarkers of nicotine exposure. Finally, they discussed other studies measuring biomarkers of nicotine exposure in long-term vapers, some longitudinally.

From these studies, NASEM concluded that:

> "There is **conclusive evidence** that exposure to nicotine from e-cigarettes is highly variable and depends on product characteristics (including device and e-liquid characteristics) and how the device is operated."

> "There is **substantial evidence** that nicotine intake from e-cigarette devices among experienced adult e-cigarette users can be comparable to that from combustible tobacco cigarettes."

In a separate chapter entitled 'Dependence and abuse liability', NASEM (21) commented that while risk and severity of 'tobacco' dependence are linked to amount of tobacco use, the correlation was moderate and dependence symptoms were also reported by occasional and less frequent tobacco users. Consistent with our 2018 report, NASEM indicated that dependence was influenced by other factors in addition to the amount of exposure. They reported that the addiction potential of tobacco products was linked to the pleasant stimuli associated with tobacco self-administration that were synergistic with the delivery of nicotine in causing dependence, such as taste, smell, and other sensations. Similarly, NASEM reported that it is likely that vaping products would cause dependence symptoms, that these symptoms would not entirely be caused by nicotine per se, although strongly influenced by nicotine. Pleasurable sensory stimuli (for example, taste, sights, smells) would also likely have a synergistic effect with nicotine on addictive potential (21). Finally, NASEM discussed how recognition of non-nicotine factors in cigarette smoking had led some experts renaming their scales (for example, the Fagerstrom Test for Nicotine Dependence was renamed the Fagerstrom Test for Cigarette Dependence) (22), an issue we return to later in this chapter.

The NASEM report gave an overview of approaches to assess dependence from vaping, stating that there was no consensus on how to assess and diagnose dependence on vaping products (21). They acknowledged that most studies (including the US Population Assessment of Tobacco and Health (PATH) study) had adapted measures of cigarette dependence to vaping products, substituting vaping products for cigarettes, and commonly drawing on the American Psychiatric Association's DSM-V (23) and ICD drug dependence classifications (24). NASEM indicated that vaping dependence could be 'operationalised as a category (for example, having at least one or more symptoms, surpassing a "clinical" threshold of 2 symptoms or more [APA 2013] or on a continuum with a score reflecting a gradient of severity of dependence from none, mild, moderate or severe'. The report also acknowledged additional measures of 'tobacco' dependence, which assessed symptoms and other domains such as motivation to use tobacco, naming the Fagerstrom Test for Cigarette Dependence, the Heaviness of Smoking Index, the Hooked on Nicotine Checklist, the Nicotine Dependence Syndrome Scale and the Wisconsin Inventory of Smoking Dependence Motives (table 1) (21).

NASEM purported that the optimum epidemiological study to assess dependence would be a long-term cohort study in a nationally representative sample which followed those initiating vaping and tracking dependence over time. NASEM acknowledged that a critical confounder in assessing vaping dependence in populations is the use of tobacco products, either concurrently, historically or prospectively. Concurrent tobacco use may not adequately be controlled for by statistical adjustments. Historical tobacco use, particularly for former regular smokers, may confound current vaping dependence measurements simply as a result of former smokers wanting to regulate their nicotine use to a certain level as they did when they were smoking. Prospective tobacco use would complicate the assessment of never tobacco or nicotine users who take up vaping and are followed up for a significant duration, as many included would be likely to go on to use tobacco products, resulting in very small samples of ‘pure’ cases of vaping dependence to study.

Additionally, NASEM recognised the contribution of laboratory ‘abuse liability’ studies. Such studies are also complicated by the fact that it would be considered unethical to expose naïve tobacco and nicotine users to vaping products, meaning that in most cases, studies are carried out with tobacco users or experienced vapers. NASEM noted that controlled behavioural conditions rather than enabling participants to use products ad libitum enable cross-product comparisons but may suffer from ecological validity considerations as they may not replicate vaping in the natural environment. NASEM commented that the outcomes would need careful consideration given withdrawal relief can be caused by products with little or no abuse liability such as some NRT products. NASEM indicated such laboratory abuse liability studies would provide supportive evidence.

Finally, NASEM recognised that clinical studies in which vaping products are provided may also contribute to understanding vaping dependence, but the ad libitum behaviours of the products involved as well as other nicotine use (such as continued smoking) may confound comparisons across conditions. NASEM indicated such clinical studies would provide ancillary evidence.

NASEM (21) carried out a systematic review of the literature addressing 3 questions:

Does use of e-cigarettes have an effect on e-cigarette dependence risk?

Is the effect of e-cigarette use on e-cigarette dependence risk weaker than the effect of combustible tobacco cigarette use on cigarette dependence?

Do e-cigarettes with certain product characteristics have stronger effects on e-cigarette dependence risk than those with other product characteristics?

NASEM identified 15 epidemiological studies on nicotine dependency and vaping products, all of which were cross-sectional: 3 used nationally representative samples (PATH), 6 online surveys not using a systematic sampling method, 2 in-person studies

using non-representative sampling and 4 laboratory-based studies. NASEM also identified an additional 9 human laboratory studies examining abuse liability outcomes and 2 relevant clinical trials. From these studies, NASEM drew 3 conclusions (21):

> "There is **substantial evidence** that e-cigarette use results in symptoms of dependence on e-cigarettes."
>
> "There is **moderate evidence** that risk and severity of dependence are lower for e-cigarettes than combustible tobacco cigarettes."
>
> "There is **moderate evidence** that variability in e-cigarette product characteristics (nicotine concentration, flavouring, device type, and brand) is an important determinant of risk and severity of e-cigarette] dependence."

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) review (25) drew on two reviews of nicotine pharmacokinetics (26, 27) which drew similar conclusions to those outlined above concerning the mode of nicotine administration.

Drawing on clinical studies in the NASEM review, COT indicated that nicotine exposure was affected by the vaping product used as well as e-liquid nicotine form and concentration, power output of the vaping product, puffing topography and vaper characteristics. COT also reviewed biomonitoring studies. Among tobacco cigarette smokers who switched to vaping products (containing nicotine), variable findings relating to plasma nicotine concentrations in users were observed. However, among long-term vaping product (containing nicotine) users, plasma levels similar to those observed in cigarette smokers were attained. COT noted that because of their more effective nicotine delivery, higher-power devices were frequently used with lower nicotine concentrations. COT cautioned however that using e-liquids with lower nicotine concentrations could cause users to increase their puffing topography in order to titrate their nicotine leading to increased exposure to other aerosol constituents. COT also noted that as nicotine absorption was pH dependent it would be affected by the e-liquid formulation.

COT noted that while the similar pharmacokinetic profile from vaping products to those of cigarettes may aid smoking cessation, it could also result in dependence in naïve users. COT also noted the role of non-nicotine factors in cigarette dependence and the complexity of cigarette dependence overall, commenting that dependence in vapers may therefore differ to that in smokers.

COT therefore concluded:

> "Experienced users self-titrate nicotine intake from Electronic Nicotine Delivery Systems (ENDS). Systemic exposure levels of nicotine equivalent to those from combustible cigarette smoking can be achieved. Factors influencing the level of nicotine exposure and retention include ENDS product type, user profile, usage parameters, e-liquid nicotine concentration, and the overall formulation of the e-liquid."
>
> "Non-users who have never been exposed to nicotine and who take up vaping would be at risk from effects of nicotine to which they would not otherwise be exposed. This also includes the risk of addiction."

5.3 Evidence from previous reports on nicotine toxicity

Having established that vaping results in nicotine exposure and some level of dependency, this section now summarises evidence on the effects of nicotine on health. As stated above, since the 1970s it has been recognised that nicotine was not the cause of the majority of the harm from cigarette smoking (5), but concerns persist about what harms nicotine causes.

Summary of our previous evidence reports

In our 2015 report (19), we reviewed the toxicity of nicotine, noting that although nicotine had been used in tobacco and nicotine replacement therapies (NRT) by thousands of millions of people, fatal nicotine poisoning was extremely rare. One possible reason for this was that relatively low doses of nicotine cause nausea and vomiting, which can deter further use. We also reported that Mayer had identified that the often-repeated claim that 'ingestion of 30-60 mg of nicotine is fatal' was found to be based on questionable self-experiments conducted in the 1890s (28). Mayer concluded that the lower limit for fatality might be more likely to be in the range of 500 to 1000mg ingested nicotine (28).

We also summarised evidence on calls to poison centres following accidental exposures and concluded that e-liquids should be in 'childproof' packaging, and we also discussed a few case studies of the use of nicotine in suicide attempts. We revisit this again in chapter 13 on poisonings, fires and explosions. Finally, we identified that vaping products released negligible levels of nicotine into ambient air with no identified health risks to bystanders.

In our 2018 report (20), we summarised evidence from the Royal College of Physicians (RCP) report which also reviewed nicotine harms (29). This concluded that short term nicotine use did not result in 'clinically significant harm' and there was no evidence of any increase in the risk of heart attack, stroke or death from the use of NRT in quit attempts. The RCP drew on the Lung Health Study (30) which, although from 2009, remains the best

study of long-term NRT use. This randomised controlled trial, which followed up NRT users for 7.5 years, reported that there was no evidence of a relationship between NRT use and cancers, whereas continued smoking was associated with developing cancer (30).

For longer term use of nicotine, our 2018 report (20) summarised the evidence for snus, a low nitrosamine smokeless tobacco product, and reported that the Global Burden of Disease Study (31) did not find sufficient evidence of a detrimental effect of snus on any outcome (including oral and pharyngeal cancer). However, we also commented that the risks of long-term inhaled nicotine separate from inhaling smoke had not been studied in humans.

We indicated that studies showing nicotine caused transient stiffening of arteries was due to acute sympathetic activation induced by nicotine through norepinephrine release (32). We also reported on a review of nicotine in vaping products on cardiovascular function which concluded that short-term use of vaping products appeared to pose low cardiovascular risk in healthy users (33), but that adverse effects may exist in people with pre-existing cardiovascular diseases, although lower than those due to smoking. This stemmed from evidence that among people who have suffered a myocardial infarction and continue to use snus, there were lower survival rates than among those who quit snus, but other factors potentially contributing to the continued snus use were not taken into account.

Our 2018 report (20) also commented on concerns about nicotine use in relation to foetal development and cognitive and additional deficits in adolescents, but that more research was needed particularly in relation to vaping product use.

The National Academy of Science, Engineering and Medicine

NASEM summarised the specific health risks of nicotine for cancer, respiratory disease and cardiovascular diseases (21).

Cancers

NASEM stated that while there was a biological rationale for how nicotine could be a tumour promoter, ‘the existing body of evidence indicates this is unlikely to translate into increased risk of human cancer' (21).

In relation to different forms of nicotine, NASEM indicated that NRT use was not associated with increased cancer rates (again citing the Lung Health Study) and studies of smokeless tobacco users showed an increase in cancer risks related to tobacco-specific nitrosamine exposure but not of other cancers. NASEM concluded that it is ‘unlikely that nicotine exposure acts as a tumour promoter to increase the risks of cancer in humans’. Based on this evidence, NASEM summarised that ‘it is reasonable to infer there is likely no

significant increase in risk of cancer from exposure to nicotine delivered by e-cigarettes' (21). NASEM conclusions relevant to vaping products and cancer are summarised in chapter 9 of this report.

Respiratory disease

NASEM also identified 3 putative pathways through which nicotine could damage the respiratory system or worsen pre-existing lung conditions: decreased viral and bacterial clearance; impaired cough; and α7 nicotinic acetylcholine receptor activity and cystic fibrosis transmembrane conductance regulator dysfunction in the airways (21). NASEM's overall findings and conclusions relevant to respiratory health are summarised in chapter 10 of this report.

Cardiovascular disease

Nicotine stimulates the sympathetic nervous system and its effects on the cardiovascular system may be due to activation of nicotinic acetylcholine receptors in endothelial, immune, neuronal and muscle cells. NASEM indicated that nicotine increases adrenal release of epinephrine and adrenergic neuron release of norepinephrine, and that heart rate and blood pressure increase regardless of the nicotine source or route of administration. Nicotine also causes a constriction of coronary blood vessels and blood vessels in the skin but a dilation of blood vessels in skeletal muscles. Acute nicotine exposure is also associated with a decrease in heart rate variability. Nicotine also impacts coronary blood flow, through constricting coronary arteries and decreasing blood flow, as well as increasing cardiac output which causes flow-mediated dilation. The report commented that while these overall nicotine effects on the sympathetic nervous system are unclear, the 'increases in heart rate, reduction in heart rate variability, and endothelial dysfunction can lead to reduced myocardial blood flow, coronary occlusion, and increased myocardial demand for oxygen and nutrients, all of which are known to be associated with increased risks of myocardial ischemia/infarction and sudden death' (21).

NASEM also reviewed other effects of nicotine on the cardiovascular system such as myocardial remodelling, arrhythmogenesis, thrombogenesis, endothelial dysfunction, inflammation and angiogenesis. Persistent sympathetic stimulation by nicotine can enhance myocardial tissue remodelling which creates heart failure. Arrhythmogenic nicotine effects are mediated through catecholamine release, which can contribute to ventricular tachycardia and fibrillation. In relation to the thrombogenic effects of nicotine, whereas animal studies have shown mixed effects of acute and long-term nicotine intake, studies of NRT and smokeless tobacco do not show increased platelet activation. Endothelial dysfunction consists of impaired flow-mediated dilation (the vasodilatory response to increased local blood flow) which is mediated by oxidative stress and chronic inflammation, and NASEM indicated that the effect of nicotine over and above the effects of powerful oxidants and pro-inflammatory agents found in cigarettes was unclear. However, endothelial dysfunction had been observed to be impaired following local

nicotine infusion or use of a nicotine inhaler. Nicotine appeared to have both anti- and pro-inflammatory effects. However, NASEM summarised that nicotine was ‘not believed to be the main determinant of an inflammatory response in smokers’ based on studies of smokers showing a significant decline in inflammatory markers after switching to transdermal nicotine, and similar inflammatory marker levels between smokeless and non-tobacco users. Similarly, nicotine was not identified as an important driver of smoke-related angiogenesis (21).

NASEM also mentioned that while nicotine can induce a more atherogenic lipid profile, cessation studies using NRT found improvement in high-density lipoproteins/low-density lipoprotein ratios and reduced dyslipidemia. NASEM also commented that nicotine-induced vasoconstriction could play a role in the progression of chronic hypertension to malignant hypertension. Finally, NASEM commented that nicotine appeared to be responsible for increased insulin resistance in smokers. The authors noted that nicotine-induced release of several hormones which were insulin antagonists could enhance insulin resistance, and that nicotine directly activated adenosine monophosphate-activated protein kinase via α7 nicotinic acetylcholine receptor effects in adipose tissue (21).

For cardiovascular disease, NASEM mention in their summary that ‘exposure to nicotine from e-cigarettes likely elevates the risk in people with pre-existing cardiovascular disease(s), but the risk in people without cardiovascular disease is uncertain’ (21).

NASEM’s conclusions relevant to vaping and cardiovascular disease are summarised in chapter 11 of this report.

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

COT reviewed toxicological data for nicotine by searching 2 databases (Scopus and PubMed) from 1 January 2008 to 29 April 2019 with literature prior to 2008 being identified from several published toxicological review of nicotine (25). This report also drew on the Mayer 2014 review which cast doubt on the widely cited lethal dose of nicotine. COT reported that poisoning mostly related to ‘accidental or deliberate ingestion or dermal exposure’. COT noted case reports describing poisonings from vaping products, most either by young children or accidental exposures, and included ‘vomiting, lactic acidosis, and in some cases, death’.

Findings on cancer were based on the aforementioned Lung Health Study, namely that this study ‘found an absence of any relationship between NRT use and lung, gastrointestinal, or all cancers over a relatively short follow-up period’. With regard to cardiovascular disease, COT reported evaluations of cardiovascular disease based on studies assessing NRT as an aid to quitting cigarette smoking, concluding that studies

were ‘mostly of inadequate quality to draw clear conclusions but have not shown evidence of serious cardiovascular events’ (25).

COT also reviewed the effects of nicotine on developmental outcomes, from exposure via parents prior to or during pregnancy or lactation and determined that the studies were not able to identify nicotine specific effects. COT also reviewed nicotine effects in adolescents and young adults but found no data on the direct effects of nicotine in humans to examine. COT commented that while brain development in humans continues to around 25 years of age, there was the potential for nicotine to have adverse neurodevelopment effects as well as negatively affecting mental health. COT concluded that, based on animal studies, particularly effects on the developing lungs, there ‘is good biological plausibility for an effect of nicotine on development’. However, they cautioned against trying to quantify the effects of nicotine in humans based on animal studies given the unclear relationship of dosing to human exposures (25).

Finally, COT also reviewed bystander studies, indicating that nicotine was emitted into ambient air from vaping products and that this could result in a risk of nicotine-related adverse health effects for some bystanders where smoking was banned.

Overall, the COT conclusions relating to nicotine health risks and vaping products were (25):

1. For people who switch from [cigarette] smoking, the risks associated with nicotine exposure from ENDs would be expected to be similar to those from the same nicotine exposures through use of [cigarettes].

2. It is thus anticipated that nicotine-related health effects could occur with long-term use of ENDs. Risks include effects on a large range of endpoints in users and their offspring.

3. Use of ENDS while continuing to smoke [cigarettes] (dual use) could potentially lead to increased nicotine exposure compared with that from [cigarette] smoking only and may increase the overall risk.

4. Bystanders are likely to be exposed to some nicotine in ambient air where ENDS are used, which may have some associated effects.

5.4 Updated evidence from the systematic reviews covered in this report

As identified in the introduction to this chapter, nicotine dependency and toxicity are important issues of concern when understanding the potential role of vaping products in reducing the harms caused by tobacco smoking.

As previously identified, nicotine dependency has implications for whether vaping products will act as substitutes for tobacco cigarettes, acknowledging that other non-nicotine factors will also play a role. The first consideration is the extent to which vaping products deliver nicotine, how that varies by product (for example, device, nicotine content, presence of salts) and in relation to tobacco smoking. To update evidence in prior reports we were therefore interested in addressing the following research question:

1. What is the nicotine exposure profile of vaping products compared with smoking and across different types of vaping products?

To address this research question, we identified studies in our review which examined the pharmacokinetics of nicotine and we describe these studies in this chapter, as well as studies assessing biomarkers of nicotine which are described in chapter 7 (biomarkers of exposure) and summarised here.

The second consideration was the health effects of nicotine, which also have implications for the health effects of vaping products. To update evidence in the prior reports, we therefore addressed this second research question in this chapter:

2. What role does nicotine play in the health risks of vaping?

To address this research question, we identified studies in our review which assessed the role of nicotine and health risks which are described in the subsequent health chapters (9 to 13) and summarised here. For both research questions, we use evidence from the systematic review carried out for this report, as summarised in chapter 2 (methods).

In addition to the first research question, we were also interested in assessing the overall dependency of vaping products, how this varied by product (for example, device, nicotine content, presence of salts), and in relation to tobacco smoking. Given the extent of the literature in this important area, it was not possible to carry out a separate systematic review of the dependency of vaping products for this report. However, we were aware of several ongoing relevant reviews, which we anticipated being published during our write-up period. Ultimately, only one review was published (34), and the findings of that review are summarised after addressing our first research question focusing on nicotine pharmacokinetic studies and nicotine exposure.

5.5 Review of nicotine pharmacokinetic studies

Drawing on the systematic review of the health risks of vaping described in the methods, we identified 20 nicotine pharmacokinetic studies from vaping product use in humans (35 to 52, 53 , 54), which are summarised in table 2. Given our review’s focus on objective vaping effects on users’ health, we extracted only the pharmacokinetic data and did not

include the subjective measures that might be associated with exposure to nicotine from vaping products.

We did not identify any randomised control trials exploring pharmacokinetic nicotine delivery profile of vaping products. Fifteen were cross-over studies (35 to 47, 53, 54) and 5 acute exposure studies (48 to 52). Three were conducted in the UK (40, 41, 53), 3 in Germany (38, 42, 49) and 14 in the US (35 to 37, 39, 44 to 48, 50-52, 54, 55). Of the included studies, 4 (39, 45, 49, 53) were funded by the tobacco industry (British American Tobacco (Investments) Limited, Imperial Brands Limited and Altria Client Services), 2 were funded by JUUL labs, inc. (35, 54) and one study (50) was not funded by the tobacco industry, but declared that an author received grants and fees from both vaping and tobacco industry (appendices: table 5).

The 15 cross-over studies had sample sizes ranging from 15 to 71 and included vaping product users, 'dual users' and/or smokers. The 5 acute studies had sample sizes ranging from 5 to 25 and included vapers, 'dual users' and/or smokers, with some studies drawing smokers' data from past research from the same researcher group and with the same design to act as controls.

Twelve studies assessed maximum plasma nicotine concentration (C_{max}), time to maximum nicotine concentration (T_{max}), and total nicotine exposure by measuring the area under the concentration-time curve (AUC), although the duration assessed for total nicotine exposure differed as indicated in table 2 (35, 38 to 41, 44 to 48, 53, 54). St. Helen and others (44) also measured plasma nicotine concentration and terminal elimination half-life of nicotine, Goldenson and others (54) assessed the rate of plasma nicotine rise, Solingapuram Sai and others (50), in addition to C_{max} and AUC, also assessed the terminal half-life of nicotine, Landmesser and others (49) assessed C_{max} and AUC only, and Spindle and others (55) assessed only AUC. Yingst and others (51, 52) assessed C_{max} and T_{max} only and Hiler and others (36, 37) and Ruther and others (42) only assessed plasma nicotine concentration.

All but 3 studies (36, 37, 51) compared vaping product use with at least one smoking condition, and 4 studies that did have smoking controls also had additional control conditions—2 nicotine gum (45, 54), one heated tobacco products (HTP) (40) and one an e-Pipe and e-Cigar (46).

Eight studies compared different nicotine concentrations in e-liquids, ranging from no nicotine vaping condition (36) to 59mg/mL nicotine conditions (35, 41). Of the studies exploring different nicotine concentrations, 4 used pod devices (35, 39, 41, 53), one used cartridge vaping products (45) and 3 used tank or modular type vaping products (36, 37, 48).

Five studies compared different types of vaping product devices, or a regular device with a modified one (38, 42, 44, 46, 51).

Five studies had other comparisons discussed in detail below (40, 49 to 51, 55).

Two studies examined nicotine delivery profile of vaping products with different flavours (47, 54) and are described further in chapter 6 (flavours).

Risk of bias in included studies

Cross-over studies

Of the 15 cross-over studies that reported on the nicotine pharmacokinetic profile after vaping product use, 12 were rated to have some concerns regarding risk of bias (35 to 39, 43 to 47, 53, 54) and 3 were rated at high risk of bias (40 to 42) according to the RoB2 risk of bias tool for cross-over studies (appendices: table 2). The 3 studies rated at high risk of bias had issues with deviations from intended interventions (40), selection of the reported result (41) and missing outcome data (42).

Acute exposure studies

Of the 5 acute exposure studies, 3 were rated at low (48, 49, 52), one at moderate (51) and one at high risk of bias (50) according to the ROBINS-I risk of bias tool for non-randomised longitudinal studies (methods: table 3). The Solingapuram Sai and others study (50) was at high risk of bias at multiple domains—due to bias in participant selection, classification of interventions and the selection of the reported results.

Findings

Cross-over studies

Ebajemito and others (53) carried out a UK study which examined 24 healthy ‘dual users’ who were exposed in a within subjects cross-over design to 6 vaping product use conditions and 2 ad libitum and controlled smoking conditions in a 9 day confinement period. The study aimed to compare pharmacokinetic nicotine delivery profile between use of protonated (nicotine salts) and unprotonated (freebase nicotine) e-liquid, use of different nicotine strengths, use of different device types, and between ad libitum and controlled puffing conditions. Two vaping products were included, the Vype ePen 3, a cartridge closed vaping system which delivers approximately twice the amount of vapour as the cartridge closed vaping system Vype ePen, which was included as a comparator in one ad libitum unprotonated 18mg/mL nicotine condition only. The 5 conditions involving the Vype ePen 3 were:

- ad libitum 18mg/mL unprotonated nicotine
- ad libitum 8mg/mL medium protonation nicotine

- ad libitum 30mg/mL high protonation nicotine
- ad libitum 12mg/mL low protonation nicotine
- a controlled condition with 18mg/mL medium protonation nicotine e-liquid

Ad libitum and controlled conditions lasted 5 minutes and the latter involved taking 10 puffs in total, one every 30 seconds. Mean C_{max} ranged from 5.82ng/mL for the vaping ePen comparator condition (with unprotonated 18mg/mL nicotine), to 18.5ng/mL for the cigarette smoking ad libitum condition. Among the ePen 3 conditions, the highest C_{max} level of 16.8ng/mL was achieved for the 30mg/mL high protonation nicotine condition. T_{max} varied from 5.7 minutes for the ePen 3 30mg/mL high protonation condition to 12.2 minutes for the ePen comparator condition.

Finally, AUC assessed over 120 minutes varied from 298ng/mL*h in the ePen comparator condition to 717ng/mL*h in the ad libitum smoking condition, with the highest level in the ePen 3 conditions again being reached in the ad libitum 30mg/mL high protonation condition. These results indicate that the cartridge type vaping product ePen 3 with 30mg/mL high protonated nicotine condition was the closest to ad libitum smoking in terms of nicotine delivery, with the T_{max} being faster than smoking a cigarette, although the C_{max} was lower.

Goldenson and others (35) involved 25 smokers (naïve vapers) in a 5 arm, randomised, within-subjects cross-over study to assess nicotine pharmacokinetics of a nicotine pod vaping device (JUUL) with different nicotine concentrations and different wicking materials for drawing the e-liquid from the reservoir to the heating coil (silica and cotton), compared with smoking. The JUUL device was tested in 4 different conditions, each involving controlled and ad libitum phases, which were:

- 59mg/mL Virginia Tobacco flavour with silica wick
- 18mg/mL Golden Tobacco flavour with silica wick
- 18mg/mL Golden Tobacco flavour with cotton wick
- 9mg/mL Golden Tobacco flavour with cotton wick

At each session, each nicotine condition was tested first with a 5 minute controlled vaping protocol (10 3-second puffs, each puff inhaled at approximately 30-second intervals) and then 5 minutes an ad libitum vaping protocol. The smoking conditions were also ad libitum and controlled (10 3-second puffs, each approximately 30 seconds apart) with participants smoking their own brand of cigarettes. C_{max}, AUC assessed over 60 minutes and T_{max} were statistically significantly higher in the smoking than the vaping product conditions for both ad libitum and controlled phases. Among the vaping product conditions, C_{max}, AUC

and T_{max} were statistically significantly higher for the 59mg/mL product than the other conditions. For vaping products, nicotine exposure increased in accordance with the nicotine concentration of devices and was highest in the 59mg/mL condition. The different wicking materials did not have a differential effect on pharmacokinetics.

Hiler and others (36) compared 33 self-reported 'dual users' of vaping and tobacco products with 31 self-reported smokers who had never used vaping products. The 4 cross-over conditions involved using a tank-type vaping product (eGo, 3.3 volt, 1000 milliampere hour (mAh) battery and 1.5 ohm (Ω) resistance dual coil) with a 70% to 30% propylene glycol (PG) to vegetable glycerine (VG) ratio of tobacco- or menthol-flavoured (chosen by participants) e-liquid with nicotine separated by over 48 hours. The 4 conditions comprised 2 vaping bouts set one hour apart using 0, 8, 18 and 36mg/mL nicotine e-liquids. No statistically significant differences in plasma nicotine concentration were reported for 'dual users' compared with baseline, when using the nicotine-free liquid, but a significant increase when using 8, 18 and 36mg/mL nicotine e-liquids. For plasma nicotine concentration among smokers, there was no statistically significant difference compared to baseline after they used the 0 and 8mg/mL e-liquids, but statistically significant increase after using 18 and 36mg/mL e-liquids. 'Dual users' had a statistically significant higher increase in plasma nicotine levels compared with the smokers when using 8, 18 and 36mg/mL vaping liquids but not for the nicotine free condition. The authors suggested that the higher levels of nicotine plasma among 'dual users' was likely due to the longer vaping puffs employed by experienced vapers, reporting a mean puff duration of 5.6 seconds among experienced vapers compared with 2.9 seconds among those who had never used vaping products before.

Hiler and others (37) included 32 self-reported vapers who were smoking fewer than 5 cigarettes per day. They employed 4 conditions separated by 48 hours and each condition involved controlled vaping product use followed by a period of ad libitum vaping product use. Each condition involved using a Kangertech Subox battery attached to a Subtank mini tank with 30%/70% PG/VG, pear-flavoured and either 3mg/mL or 8mg/mL nicotine strength e-liquid. Participants took 10 puffs with 30-second inter-puff intervals, followed by one hour non-use and then one hour of ad libitum vaping. For each nicotine condition, there were 2 power and coil resistance conditions. These were either a 40.5 watt (W) power and 0.5 Ω ('sub-ohming') or 13.5 W power and 1.5 Ω. For the 2 conditions with 40.5 W and 0.5 Ω with 3 and 8mg/mL, and the 13.5 W and 1.5 Ω 8mg/mL condition, plasma nicotine concentrations were statistically significantly higher in the ad libitum conditions than in the controlled conditions, which were statistically significantly higher than baseline. There was no statistically significant difference in plasma nicotine concentrations between the controlled condition and baseline in the 13.5 W and 1.5 Ω 3mg/mL nicotine condition, but the ad libitum use phase showed a statistically significant plasma nicotine increase compared with baseline. For controlled use, there was a statistically significantly higher increase in the 0.5 Ω, 8mg/mL condition compared with the other conditions. For ad libitum, there was a statistically significantly higher increase in plasma nicotine in the

40.5 W and 0.5 Ω 8mg/mL condition compared with the other 8mg/mL nicotine condition and the 1.5 Ω 3mg/mL conditions. There was no statistically significant difference after ad libitum use in the 0.5 Ω 8mg/mL and 0.5 Ω 3mg/mL nicotine conditions. Statistically significant higher nicotine exposure was observed in the 40.5 W and 0.5 Ω conditions than the 13.5 W and 1.5 Ω conditions. The authors also noted that vapers using the lower nicotine concentration e-liquids (3mg/mL) took longer, larger in volume and more frequent puffs, and that 'sub-ohming' resulted in more vaping liquid being consumed than when using 1.5 Ω coil resistance conditions.

Mallock and others (38) included 17 vapers and 15 smokers. The study compared the impact of a 'modified' JUUL wick, that purportedly allowed for greater aerosol generation, with the 'initial' JUUL version. Participants who had been using vaping products were invited to participate in both vaping conditions and those who had been smoking were invited only to smoking condition. The first condition comprised 5 minutes of controlled vaping (3-second puffs, each puff inhaled every 30 seconds) using the 'initial' JUUL vaping product with 18mg/mL nicotine salt pod. The second condition involved the same regimen, but with the 'modified' JUUL vaping product with 18mg/mL nicotine salt pod. The third condition involved smoking Marlboro Red tobacco cigarettes by taking the same number of puffs over 5 minutes. The authors reported that differences between the 2 JUUL conditions were small and non-significant for all pharmacokinetic parameters. C_{max} and AUC in the vaping conditions were approximately 40% to 50% smaller than after tobacco smoking. The study authors concluded that the JUUL products delivered lower levels of nicotine compared with the cigarette condition, but that there were few differences between the 'initial' and 'modified' wick, while acknowledging that the differences in wick performance might be more noticeable after a longer period of use.

O'Connell and others (39) conducted a randomised open-label, cross-over clinical study with 15 smokers (naïve vapers) over 6 conditions including a controlled smoking condition. The study used the pod type vaping product Myblu with 350 mAh battery and 1.3 Ω resistance coil and tobacco flavour and compared a freebase 25mg/mL condition with a salt-based 16mg/mL, 25mg/mL and 40mg/mL conditions. An additional condition used a salt-based 48mg/mL blu PRO tank vaping product. Each condition involved 10 3-second puffs, each taken 30 seconds apart. The tobacco cigarette resulted in a C_{max} of 17.8 ng/mL which was statistically significantly higher than C_{max} values in all the vaping conditions, except the salt-based 40mg/mL pod condition. The range for the vaping conditions was between 5 ng/mL for the freebase 25mg/mL pod condition and 10.3 ng/mL for the salt-based 40mg/mL pod condition; use of the 48mg/mL salt-based tank vaping product resulted in 4.9ng/mL C_{max}. There were no statistically significant differences between groups in the time to maximum nicotine concentration (T_{max}). The AUC, assessed over 30 minutes, was highest for the tobacco cigarette, statistically significantly higher than the AUC for all the vaping conditions except the salt-based 40mg/mL pod condition. The study authors concluded that all vaping products on average delivered less nicotine than

cigarettes, although the time to reach the peak nicotine concentration in the bloodstream was similar across all the products to tobacco cigarettes.

A study by Phillips-Waller and others (41), which was carried out in the UK and involved 18 ‘dual users’ who vaped daily and smoked occasionally, aimed to assess the pharmacokinetic nicotine delivery profile of a pod type vaping product (JUUL) with 2 different nicotine concentrations and a tobacco cigarette when used by experienced vapers. The study assigned participants to 3 arms in a within-subject cross-over design where they ad libitum used products for 5 minutes. They were:

- the pod vaping product JUUL with 20mg/mL nicotine salts and Golden Tobacco flavour
- the same JUUL with 59mg/mL nicotine salts and Virginia Tobacco flavour
- own brand cigarettes

The results of a fourth condition, which involved 7 of these participants using different vaping products with tobacco flavour and 16 to 48mg/mL nicotine e-liquids, were not reported.

Pharmacokinetic findings were compared for JUUL 20mg/mL with the JUUL 59mg/mL condition and the smoking condition separately. Median C_{max} was statistically significantly higher in the 59mg/mL JUUL condition and in the smoking condition compared with the 20mg/mL condition. Median T_{max} was not statistically significantly different between the 3 study conditions. AUC assessed over 30 minutes was statistically significantly higher for the 59mg/mL JUUL product and smoking conditions compared with the 20mg/mL condition. Overall, the 20mg/mL JUUL delivered nicotine slower and at statistically significantly lower levels than the 59mg/mL JUUL product and cigarette smoking.

Ruther and others in a study from Germany (42) included 20 participants, 9 of whom were using vaping products and 11 smoking. The study compared plasma nicotine concentrations from exposure to a cartridge vaping product (American heritage, Vype or Blu) with strawberry/mint flavoured and 18mg/mL nicotine strength e-liquid with 18mg/mL nicotine concentration tank vaping products (Aspire/Joytech eGo C2, 650 mAh battery, 1.8 Ω) condition. These were compared with plasma nicotine concentrations in a separate sample of smokers when smoking tobacco cigarettes (Marlboro Red). The authors measured nicotine plasma concentrations after 5 minutes of controlled use, which included 10 4-second puffs with 26 seconds between puffs for the vaping products, and 10 2-second puffs with 28 seconds between puffs for the cigarette. Nicotine exposure in both the cartridge and tank conditions were statistically significantly lower than the plasma nicotine concentration after smoking, but there was no statistically significant difference between the 2 vaping products.

A US study by Spindle and others (43) included 30 healthy vapers or 'dual users' and examined the impact of different PG and VG ratios on nicotine absorption. Participants took part in 2 sessions, 60 minutes apart, where they used a tank type vaping product (eGo 3.3 V battery with 1.5 Ω dual-coil, 7.3 W) with 18mg/mL nicotine e-liquid taking 10 puffs, each 30 seconds apart. The 4 conditions differed in PG/VG ratio: 100% PG, 55% to 45% PG/VG, 20% to 80% and 2% to 98%. Total nicotine exposure (AUC) at one hour after the initial puffing session was highest for the 100% PG condition, which was statistically significantly higher than for the 20%/80% or the 2%/98% PG/VG conditions (results for 55%/45% PG/VG ratio were not reported). Given this was one of few studies to examine PG/VG effects, we also note here the topography and satisfaction findings. The authors reported that PG/VG ratio affected participants puffing topography. When using the 100% PG vaping liquid, participants took shorter and smaller puffs compared with other PG/VG conditions and subjectively rated this e-liquid as least satisfying. The study concluded that an e-liquid containing only PG delivered more nicotine than a mix of PG/VG in vaping liquid.

St Helen and others (44) in a 2 arm, counterbalanced cross-over study compared vaping products and cigarettes among 'dual users' in the US. The vaping arm comprised one vaping condition, wherein the 36 participants used their own vaping products, taking 15 puffs on a cartridge or pod or 10 puffs on a tank or mod vaping products to deliver around 1 mg of nicotine as when smoking a cigarette. The puffs were 30 seconds apart and flavours and strengths varied. The second condition comprised smokers smoking their own brand of cigarette to completion. C_{max} and plasma nicotine concentration were statistically significantly higher in the smoking compared with the vaping condition. T_{max} was also statistically significantly faster in the cigarette condition compared with the vaping product condition. AUC assessed over 240 minutes was also significantly higher for the smoking condition compared with the vaping condition. The study also measured the terminal elimination half-life of nicotine, finding that the half-life from smoking was statistically significantly shorter than for vaping. The authors also compared nicotine exposure between different types of vaping products and concluded that users of vaping products with variable power tanks were exposed to highest levels of nicotine compared with users of disposable, cartridge, pod or tank-type vaping products. Overall, the study concluded that nicotine intake and exposure are lower after a single session of using a vaping product compared with smoking a cigarette.

Stiles and others (45) examined nicotine exposure of a menthol-flavoured pod vaping device (Vuse Solo) containing 3 different concentrations of nicotine (14, 29, and 36mg/mL), compared with participants smoking their own brand menthol cigarettes or using 4mg nicotine gum. The study recruited participants, who smoked menthol cigarettes and were not regular vapers, and randomised them to one of 10 investigational product sequences. This included an at home phase to accustom the participants to the products followed by the clinic sessions which involved 10 minutes of ad libitum use of the vaping product or smoking one cigarette, or up to 30 minutes of ad libitum use of 4mg nicotine

gum. Nicotine exposure (AUC assessed over the first 15 minutes) was statistically significantly lower in all the vaping conditions compared to smoking and statistically significantly higher than 15 minutes after the nicotine gum use. Longer-term nicotine exposure (AUC assessed over 6 hours) was again significantly lower in all vaping conditions than the smoking condition, but also statistically significantly lower than the nicotine gum condition. Maximum plasma nicotine concentration (C_{max}) was also statistically significant lower in the vaping conditions compared to smoking, statistically significantly lower for 14mg/mL nicotine condition than the nicotine gum, but not statistically significantly different between the nicotine gum and 29mg/mL or 36mg/mL vaping conditions. T_{max} was statistically significantly longer for the vaping conditions than the smoking conditions but shorter than the gum condition. This study indicated that the vaping product conditions resulted in lower peak and overall nicotine exposure than smoking; speed of delivery for vaping was slower than smoking but faster than after nicotine gum use, although overall exposure to nicotine from vaping was lower than after use of the 4mg nicotine gum.

Voos and others (46) carried out a within-subject randomised cross-over trial in which they compared 7 conditions with 18 participants who smoked. Each vaping product was used for 20 puffs, with 30 seconds between puffs. The study included:

- a disposable vaping product (v2, 2.9 Ω, 3.96 V) with 18mg/mL nicotine e-liquid
- a cartridge model (Green Smoke, 3.4 Ω, 3.8 V) with 24mg/mL nicotine e-liquid
- a tank model (eGO, v2, 3.3 Ω, 4.14 V) with 24mg/mL nicotine e-liquid
- a modular vaping product (iTazte, VTR vaporizer, 2.6 Ω, 6.1 V) with 24mg/mL nicotine e-liquid
- an e-cigar (Cuvana) with 18mg/mL nicotine e-liquid
- an e-pipe (Smoktech, 2.5 Ω, 6.0 V) with 24mg/mL nicotine e-liquid

These were all compared with smoking ad libitum the participants' own brand cigarettes. All vaping devices produced significantly lower nicotine C_{max} than the cigarette, but there were differences among the vaping products with the lowest in the e-cigar, e-pipe and disposable vaping products, and the highest in the tank and modular devices. T_{max} was statistically significantly longer than smoking for the disposable, cartridge, mod, and e-pipe conditions; there was no statistically significant differences between the tank and e-cigar compared with smoking.

AUC levels assessed over 10 minutes were all statistically significantly lower in the vaping conditions than the smoking condition; among the vaping products the lowest values were reported in the e-cigar and disposable conditions, the largest in the modular vaping

product condition. Total nicotine absorption also varied among vaping conditions. AUC levels assessed over 120 minutes were statistically significantly lower in the disposable, cartridge and e-cigar conditions compared with smoking; there was no statistically significant differences between the tank, modular or e-pipe conditions compared with the smoking condition.

The authors also reported a direct relationship between overall nicotine delivery and subjective amount of relieved urge to smoke with vaping products that delivered higher C_{max} and AUC levels relieved urge to smoke better. However, no direct relationship was found between speed of nicotine delivery (T_{max}) and subjective speed of relief of urge to smoke (46).

A study by Phillips-Waller and others from the UK in 2021 (40) compared pharmacokinetic profiles of using the pod (JUUL), tank (KangerTech EVOD) and modular (Innokin iTaste MVP, 4.8 V) type vaping products or smoking a cigarette with that of a heated tobacco product (IQOS). The study did not compare vaping and smoking conditions, so its findings are relevant only for comparing nicotine delivery from vaping products with nicotine delivery from HTP. They exposed 22 ‘dual users’ to 5 minutes ad libitum use of a 59mg/mL nicotine salt tobacco-flavoured pod (JUUL), 20mg/mL nicotine tobacco-flavoured e-liquid tank or modular vaping products (KangerTech or Innokin), their own tobacco cigarettes or a single tobacco-flavoured heated tobacco product. The highest median C_{max} was after vaping the pod vaping product with 59mg/mL salt-based nicotine, which was statistically significantly higher than the HTP condition that also had the lowest C_{max}. There were no statistically significant differences in C_{max} between the tank vaping product or smoking conditions when compared with the HTP. The tank and modular vaping conditions and ad libitum smoking conditions were significantly slower in nicotine delivery than the HTP condition, whereas there was no statistically significant difference in T_{max} between the pod vaping 59mg/mL nicotine condition compared with the HTP. The AUC was assessed over 30 minutes and was statistically significantly higher in the pod vaping 59mg/mL nicotine condition and the smoking condition than the HTP. The authors concluded that ad libitum HTP use delivers less nicotine than smoking or pod vaping 59mg/mL nicotine conditions, but similar nicotine levels compared with tank or modular vaping product use with 20mg/mL nicotine e-liquid.

Two cross-over studies, Voos and others (47) and Goldenson and others (54), assessed the effect of vaping e-liquids with different flavourings on nicotine delivery, so findings of these studies are described in the chapter on flavours.

Acute exposure studies

An acute exposure study by Baldassarri and others (48) recruited 7 participants, 4 daily vapers and 3 daily cigarette smokers. The vapers underwent 2 conditions, with a controlled puffing protocol of 10 puffs, each puff every 30 seconds, for 5 minutes, of either an eGo tank vaping product with either 8mg/mL nicotine or 36mg/mL nicotine e-liquid. The

smokers used the same protocol to smoke a tobacco cigarette (Camel, Turkish and domestic blend). However, the between-subjects design means that the cross-product comparisons were confounded, and hence not discussed further here. There was no statistically significant difference between the 2 vaping conditions for all outcomes reported (C_{max}, T_{max} and AUC measured for 60 to 90 minutes).

Landmesser and others (49) recruited 20 exclusive vapers and 5 daily smokers in their proof-of-concept study. This aimed to detect absorption of labelled (by using stable isotope ingredients) and unlabelled nicotine after vaping and smoking. Here we report only overall levels of nicotine exposure, as reported in the study. The first vaping set-up comprised 10 out of 20 vapers taking 10 vaping sessions of 10 4-second puffs, each taken 30 seconds apart, using a tank type vaping product set to 10 W power (Eleaf iStick TC 40W, Aspire Nautilus mini 1.8 Ω tank system) with 12mg/mL nicotine and 50%/50% PG/VG ratio. The second included the remaining 10 exclusive vapers who used the same tank type vaping product set to 18 W power with the same vaping liquid for 10 sessions. The third condition included smokers, and each of them smoked 10 non-filtered cigarettes (10 mg tar, 0.32 mg nicotine, 10 mg carbon monoxide). In general, overall C_{max} and AUC for smoking was higher than after both vaping conditions, and use of the vaping product set to higher power (18 W) delivered more nicotine than when it was set to lower power (10 W), although these differences were not tested for statistical significance.

A study by Solingapuram Sai and others (50) used positron emission tomography (PET) brain imaging technology to compare how brain nicotine kinetics differed after using vaping products and smoking cigarettes. The study was rated at high risk of bias on multiple domains, which should be considered when interpreting its findings. The study included 17 vapers or ‘dual users’, 8 of whom were currently smoking, 8 were former smokers and one had never smoked. The vaping condition comprised one standardised puff of a tank model vaping product (V2 EX Blanks refillable cartomizer with a programmable air syringe pump) with 12mg/mL nicotine concentration and 80%/20% PG/VG ratio e-liquid which was mixed with 11C-nicotine for PET after vaping. The smoking condition involved a single standardised puff of a cigarette with 11C-nicotine for PET after smoking. The C_{max} for vaping was 30.4% lower than in the smoking condition and the AUC 28.9% lower for vaping than for smoking. The C_{max} when vaping was 24.6% higher for women than for men, a difference that was also present in the smoking condition.

The first of 2 studies from Yingst and others (52) included 14 experienced vapers, 4 of whom vaped their own cartridge vaping product for 30 puffs, each puff 20 seconds apart, for 10 minutes with 12 to 24mg/mL nicotine e-liquid, and 10 who had the same puffing regime using their own tank or modular type vaping product with 12 to 20mg/mL nicotine e-liquid. These were compared with smokers from a separate study (56) who smoked one of their own cigarette ad libitum. Authors concluded that smoking a cigarette delivered statistically significantly higher levels of nicotine (C_{max}) and faster (T_{max}) compared with both vaping groups combined. C_{max} was higher in the vaping tank or modular group

compared with the vaping cartridge group, but there was no significant difference between these 2 groups for T_{max}.

Another acute exposure study by Yingst and others (51) included 6 participants, all of whom vaped pod vaping devices, and aimed to characterise nicotine absorption among experienced pod vaping product users. Participants were asked to take 30 puffs, each puff 20 seconds apart, using their own vaping devices. Blood samples were taken at 1, 2, 4, 6 and 8 minutes. The pod devices included JUUL or Ziip pods of different flavours (mango, strawberry lemonade and menthol). The authors reported the mean C_{max} (28.6ng/mL) and the mean T_{max} (8.7 minutes) and concluded that pod vaping products can deliver higher nicotine concentrations faster than the nicotine boosts obtained by cartridge and tank or modular type vaping devices, as reported in their previous study (52). Nevertheless, the pod vaping condition included more than 2 times higher e-liquid nicotine concentration than the cartridge, tank or modular vaping conditions.

Summary of pharmacokinetic nicotine delivery profile of vaping products

Peak (C_{max}) and overall (AUC) nicotine exposure

Studies that included smoking as a control condition and used controlled vaping and smoking sessions concluded that vaping products, regardless of their type or e-liquid nicotine concentration, expose users to significantly lower peak (C_{max}) and total (AUC) nicotine levels than smoking a cigarette (35, 38, 39, 41, 42, 4446, 49, 50, 52, 53) (table 2). Although studies used different durations for the total nicotine exposure when measuring the AUC, the peak nicotine concentration tended to be directly associated with overall nicotine exposure. One study (36) also reported that overall exposure to nicotine depends on users' vaping experience. In this study, experienced vapers attained higher plasma nicotine levels by drawing longer puffs on the same vaping product than smokers who were naïve to vaping. This study did not have a smoking condition for comparison.

In addition, another study (37) demonstrated that in ad libitum conditions experienced vapers tend to take longer and larger puffs when using lower nicotine concentration e-liquid, which allow them to attain higher plasma nicotine concentrations. Another study using ad libitum conditions also found similar peak and overall nicotine exposures between using a 59mg/mL protonated nicotine pod vaping product and cigarette smoking (41).

Effect of e-liquid characteristics on nicotine delivery

Findings from the included studies that explored controlled vaping sessions suggest that peak and overall nicotine exposure increase in a dose-dependent manner dependent on the nicotine concentration in vaping liquids (35, 41, 53). In ad libitum vaping conditions, experienced vapers using lower concentration nicotine e-liquids might adjust their puffing

regime and increase e-liquid consumption to compensate for lower nicotine exposure (37). One study reported that e-liquids with nicotine salts (protonated e-liquid) delivered significantly higher peak and overall nicotine levels to users than freebase nicotine e-liquids (unprotonated e-liquid) (53). Another study explored how the PG/VG ratio of e-liquids affects nicotine delivery and concluded that e-liquids composed of only PG deliver significantly more nicotine but are also perceived as less satisfying to users than e-liquids with a PG/VG mixture (43) (table 2).

Effect of vaping device characteristics on nicotine delivery

In general, studies that explored different types of vaping devices concluded that tank and modular type vaping products expose users to significantly higher levels of nicotine than disposable or cartridge type vaping products (42, 46, 52) (table 2).

One acute exposure study suggested that pod vaping products might deliver higher levels of nicotine than cartridge, tank or modular vaping products (51), but this comparison was confounded by higher e-liquid nicotine concentration in the pod compared with the other types of vaping products.

One cross-over study concluded that variable-power tank and modular type vaping products deliver significantly higher nicotine levels than vaping products without a variable-power function (44), while another study reported that the same modular type vaping product delivers more nicotine under a higher than lower power setting (49).

One study concluded that use of vaping products with a coil below 1 Ω (‘sub-ohming’) delivered higher nicotine levels to users compared with vaping products that have higher coil resistance than 1 Ω (37).

Another 2 studies explored how wicking material, which draws the e-liquid from the reservoir to the heating coil, affects nicotine delivery in pod vaping products and reported no significant effect of the wicking material on peak and overall nicotine exposure to users (35, 38).

Time to peak nicotine delivery (T_{max})

Findings regarding time to peak nicotine delivery were mixed (table 2). One study showed that vaping products with higher e-liquid nicotine concentrations deliver peak nicotine levels faster than vaping products with lower e-liquid nicotine concentrations (41). One study found no difference in time to peak nicotine concentration after vaping and smoking sessions (39), and 3 studies reported that vaping delivered peak nicotine concentrations slower than smoking a cigarette (44, 45, 52). However, the type of products and nicotine concentrations varied across the studies. Indeed, one study suggested that time to peak nicotine concentration might depend on the vaping product type, reporting that disposable, cartridge and modular vaping products delivered peak nicotine levels slower than smoking,

while the time to peak nicotine concentration after using a tank type vaping product and smoking a cigarette did not differ (46).

Protocols for vaping product pharmacokinetic studies

The reviewed studies explored many variables that influence the nicotine delivery of vaping products. These included:

- participants' vaping experience
- type of vaping exposure (ad libitum versus controlled)
- vaping product type
- composition and setting of a vaping product
- e-liquid characteristics (for example, nicotine concentration, nicotine protonation, PG/VG ratio, flavourings)

Other subjective effects were also measured although these were outside of the scope of our review.

All these variables are important, but some standardisation of these variables would improve comparison of findings. While most pharmacokinetic studies compared nicotine delivery after acute standardised vaping sessions (10 puffs, one taken every 30 seconds), even greater standardisation would help, such as controlling the puff durations, as this would facilitate direct comparisons across different products and e-liquid characteristics. However, more ad libitum vaping sessions over a longer period of time are also needed to account for participants' vaping experience and idiosyncratic puffing behaviours. These longer-term pharmacokinetic ad libitum studies could also help assess vaping dependency as discussed below. In general, the heterogeneity of included pharmacokinetic studies identified different factors that define nicotine delivery, but further research in this area should clarify how vaping product users adjust their vaping behaviour over longer periods of time and in relation to their vaping dependency.

5.6 Systematic review on exposure to nicotine and its metabolites

This section draws on the systematic review of vaping health effects described in the methods and the review of studies that reported on vaping association with exposure to nicotine and its metabolites outlined in chapter 7 (biomarkers of exposure to nicotine and potential toxicants).

We identified and reviewed 60 studies (only 5 from the UK) examining nicotine and nicotine metabolites, including 5 meta-analyses of nicotine and nicotine metabolites among at least weekly vapers and smokers. Most of the studies assessed short- to medium-term exposure to vaping products, the longest duration of exposure assessed was 2 years. In summary, the findings detailed in chapter 7 build on the evidence reviewed thus far showing generally lower acute exposure to nicotine after short-term use (up to 7 days) of vaping products compared to smoking, but similar exposure to nicotine over medium- to longer-term duration studies (longer than 7 days). This indicates that with experience people who vape can achieve similar levels of nicotine exposure to when they were smoking cigarettes. There were differences in nicotine exposure across devices, with higher exposure being associated with tank and modular vaping devices. Although assessed in few studies, there was evidence of compensatory puffing behaviour to achieve preferred nicotine levels when using lower nicotine strength liquids, and in one longitudinal study this was evident among vapers who reduced their e-liquid nicotine concentrations over time, the reasons for which were unknown.

5.7 Dependency on vaping products

This section supplements the above data with a discussion of dependency on vaping products and factors affecting this. In reviewing earlier reports, we noted that there is no consensus on the optimal scale to assess dependency on vaping products. We also discussed factors in addition to nicotine that affect the dependency of different nicotine and tobacco products, which led to some experts renaming their scales (22). Experts have therefore suggested that dependency should be assessed differently for different products (57), although they acknowledge this makes cross product comparisons very difficult. Additionally, for those using multiple products, it would be helpful to have an assessment of overall dependence.

In 2017, a US Tobacco Centre for Regulatory Science Measurement Workgroup considered measures of vaping product dependency (58). Building on the very helpful list of measures and scales of dependency reviewed by Bold and others (58), we have listed these and expanded on them in table 1. Bold and others (58) provided initial guidance on measuring dependence on vaping products. They recommended 10 dependence constructs be considered as measures of vaping product dependency. They are:

1. Quantity and frequency of use.

2. Tolerance (which could include measures additional to quantity and frequency such as increasing nicotine concentration, changing device characteristics or e-liquid constituents).

3. Perceived benefits (for example, helps you feel better, makes experiences more enjoyable).

4. Withdrawal symptoms.

5. Cravings or urges to use.

6. Use despite harm.

7. Impaired control (for example, difficulty reducing or limiting use).

8. Automaticity (for example, reaching for a vaping product without thinking about it).

9. Preference over competing rewards.

10. Sensory dependence (for example, throat hit, emission of thick vapour clouds).

Bold and others (58) recommended the Patient Reported Outcomes Measurement Information System (PROMIS) Nicotine Dependence Item Bank which incorporated many, but not all, of the domains they recommended to be used in assessing dependency. A subsequent study (59) assessed a modified version of PROMIS, the E-cigarette Dependence Scale (EDS) and demonstrated it had strong psychometric properties for assessing e-cigarette dependence with a 4-item questionnaire providing an efficient assessment. An additional study from the US (60) used PATH wave 1 data to assess whether responses to dependence symptom questions mapped onto a common ‘latent dimension’ of dependence severity for various products. This resulted in a validated 16-item cross-product dependence Tobacco Dependence (TD) index. We have included these measures in table 1 which illustrates the plethora of measures currently being used. One discriminating factor might be the extent to which a dependency measure or scale predicts vaping cessation. For example, 2 studies (61, 62) have demonstrated that The Penn State Electronic Dependence Index (PSECDI) is predictive of quitting vaping in long term vapers and ‘dual users’ respectively. The second study by Piper and others (61) also showed that PSECDI was strongly correlated with 2 further vaping dependence scales (the e-cigarette Fagerstrom Test of Cigarette Dependence (e-FCTD) and the e-cigarette Wisconsin Inventory of Smoking Dependence Motives (e-WISDM)) which were also predictive of quitting vaping.

However, others have argued that we need to look at specific constructs of vaping products which are important for smokers who switch from cigarettes, rather than predicting vaping cessation. The key consideration therefore is whether the various dependency measures for vaping are predictive of stopping smoking. Abrams and others, for example, suggested that there is a ‘sweet spot’ in relation to balancing appeal, satisfaction and toxicity in relation to different nicotine products (8). More recently, Palmer and others also referred to the growing complexity of the nicotine market, requiring an appraisal of dependency alongside harms in relation to conceptualisation and treatment of ‘tobacco use disorder’ to include less harmful nicotine delivery systems (63).

As discussed previously, vaping products that deliver sufficient nicotine quickly are likely to have a higher dependence potential, more likely to help smokers to stop smoking but also increase uptake. Hence a further consideration is for whom dependency is being assessed. If a smoker uses vaping to stop smoking, then it is likely that they will transfer their dependency from smoking to vaping. Even if vaping some products was found to result in higher dependency than on smoking (going against the evidence to date), what value should be put on this? If the product is less harmful than smoking, then heightened dependence is not optimal, but overall health risks will be reduced, even with much higher levels of use. Nevertheless, research focusing on the different topography profiles of tobacco smoking (successive intensive bursts caused by smoking whole cigarettes which cause nicotine peaks and troughs) and vaping profiles (intermittent puffs taken frequently or occasionally throughout the day) and their associations with nicotine levels and dependency assessments would be informative. This would enable guidance to be given to vapers about optimum use of products so that they minimise the risk of compensatory behaviours (risking higher exposure to potential toxicants) but also minimises the risk of inadvertently higher dependency on vaping.

However, focusing research on dependency on nicotine-naïve individuals is likely to be of greater value. Research is focusing specifically on how best to assess vaping dependency in adolescents (for example, Vogel and others (64)). Alternative measures could also be used to assess dependency in nicotine-naïve individuals, such as the ‘conversion rate’ from initial experimentation to daily use for different products. For example, Birge and others (65) examined this for cigarette smoking. Their review and meta-analysis found that about two-thirds of non-smokers who experimented with cigarettes went on to smoke daily (65). However, this assessment does not appear to have been done for vaping products and may not be possible until sufficient cohorts of long enough duration can be established. Survey data described in chapter 3 (vaping among young people) indicated that those who smoked reported experiencing higher perceived levels of addiction, urges to smoke and frequency of urges, compared with these experiences for vaping among those who vaped. These issues were explored using ITC data from 2017 to 2019 in England, Canada and the US elsewhere (66).

Overall, however, there is still no consensus on the optimum way of assessing vaping dependency and, in comparison with tobacco smoking and other nicotine products, and across different user groups (for example, adults and youth) but this is a very active area of research. It is, however, noteworthy that most of the research on assessing dependency is being carried out in the US. Having a plethora of measures and scales to assess dependency is unhelpful, and an international consensus on how best to assess dependency on vaping products and with whom would accelerate progress in this important field. Finally, identifying studies which collected dependency data at baseline and follow-ups is also difficult, given these data are often either not referred to or included in abstracts.

One recently published systematic review by Gades and others (34) addressed the research question ‘how does nicotine concentration and/or flavour affect measures of abuse potential and appeal of e-cigarettes for adult current and former cigarette and e-cigarette users?’ We briefly summarise this study here focusing on findings related to nicotine, and the interaction between nicotine and flavours; findings related only to flavours are discussed in chapter 6 (flavours).

Both human and animal studies were included. Outcomes relating to abuse potential and appeal covered:

- dependence
- pharmacokinetics
- pharmacodynamics
- preference or choice
- self-administration
- intra-cranial self-stimulation (ICSS)
- subjective responses
- sensory ratings

Overall, 41 studies were identified addressing nicotine concentrations (8 studies were also included in our review and 16 studies were 2017 or earlier and may have been included in our summaries of evidence from earlier reports). Twelve of the studies were epidemiology or survey studies and the authors concluded that these studies suggested that higher nicotine containing vaping products were associated with higher plasma nicotine levels, dependence, greater duration of use and complete switching. Five animal studies were identified, and these generally supported the epidemiology or survey study findings indicating that higher nicotine doses were more reinforcing and had higher abuse potential, consistent with the hypothesis that smokers may need a more rewarding experience to switch completely; these studies also identified that very high doses were aversive. Fifteen experiments and 9 clinical trials with humans also indicated that higher nicotine concentrations were associated with higher plasma nicotine levels as well as greater relief of craving and withdrawal symptoms, greater dependence, increased use and better substitution for cigarettes.

In relation to the effect of interactions between nicotine and flavours on abuse potential and appeal measures, 15 studies were identified including 3 animal studies (3 studies were also included in our systematic review, and 5 were dated 2017 or earlier). Three

studies addressed nicotine concentration and different flavours and reported interactions between these characteristics on outcomes including interest in trying, perceived smoking cessation efficacy, smoking urges and ratings of pleasantness or dislike for the products; these interactions applied for nicotine-containing and nicotine-free vaping products. The remaining studies addressed the effect of sweet and cooling flavours on the harshness of nicotine or appeal reduction of higher nicotine concentrations, with mixed findings. The review authors suggested that the inconsistency of the findings could be due to flavours chosen, the sweetness level of the e-liquid, smoking history of the vapers, and whether they were using the vaping product to stop smoking.

Overall higher nicotine concentrations in vaping products appeared to increase abuse liability and appeal, and hence increase smoking cessation. The authors cautioned that imposition of limits on nicotine concentration to protect youth who have never smoked, might inadvertently reduce smoking cessation in adults.

5.8 Systematic review on the health effects of vaping

In this section we address the third research question and the role that nicotine may play in the health risks of vaping. Evidence from the systematic review on the health effects of vaping is summarised in subsequent chapters, and in general the studies did not enable us to draw conclusions on the specific health effects of nicotine in vaping products. The small minority of people who vape non-nicotine e-liquids is likely to limit research aiming to study the long-term effects of nicotine for people who use vaping products for longer periods of time.

In chapter 8 (biomarkers of potential harm to health cutting across several diseases), we identified and reviewed studies that assessed biomarkers of potential harm associated with oxidative stress, inflammation, endothelial function and platelet activation. Most of the studies assessed acute vaping effects only, and we were not able to isolate the effects of nicotine in vaping products from other factors (for example, PG/VG ratio, flavourings, vaping device type, puffing behaviour) that can influence these biomarkers. Similarly, in the overview of human studies in chapter 9 (cancer) and chapter 10 (respiratory diseases), we were unable to isolate the effects of nicotine from vaping or smoking.

Some studies that were included in chapter 11 (cardiovascular diseases) assessed cardiovascular biomarkers in humans through non-nicotine vaping as well as nicotine vaping, but the heterogeneity of the included studies limits conclusions. Whereas meta-analyses of cross-over studies from vaping nicotine and non-nicotine products for heart rate and blood pressure found no differences, these findings were not confirmed by other studies that could not be meta-analysed due to heterogeneity in study designs. The findings were more consistent in relation to nicotine effects on pulse wave velocity, where nicotine in vaping products did appear to be implicated at least in acute exposure studies.

In chapter 13 (poisonings, fires and explosions), we summarise evidence from the included studies on nicotine poisoning. Only 2 out of the 22 included case studies were from the UK. A common drawback of the included studies was that very little detail was given on the dose of nicotine ingested, which limits what we can learn from these cases.

As it is difficult to isolate nicotine effects on human health when vaping or to compare these effects between vaping and smoking, animal and cell studies can be illuminating as they can more easily differentiate nicotine-specific effects. Although the generalisability of these findings to humans is very unclear, we include a summary of these studies in relation to nicotine effects for completion.

5.9 Synthesis of animal and cell studies

In cell and animal studies, the addition of nicotine to vaping aerosol exposure elicited variable effects that were also dependent on other vaping product constituents and the exposure regime.

In relation to dependency issues, being widely distributed throughout the brain, nicotinic acetylcholine receptors were found in several crucial regions associated with different cognitive processes and reward systems. Animal studies, described in chapter 12 (other diseases), revealed that exposure to vaping products containing nicotine induced expression of nicotinic receptors α4/β2 and α7 nicotinic acetylcholine receptors and caused alterations in neurotransmitter levels within mesocorticolimbic areas, which may contribute to initiation and development of nicotine dependence (67 to 69).

Turning next to potential risks to health from nicotine, some cell studies reported that nicotine through vaping product exposure produced differential effects on cytotoxicity (70 to 73), however, the results were inconclusive due to variations in responses by nicotine concentration, different flavourings and across cell lines. Additionally, a study by Zahedi and others (74) concluded that nicotine alone induced adverse cellular responses in mouse neural stem cells, including:

- autophagy
- dysfunction and mitochondrial hyperfusion
- oxidative stress
- mitochondrial DNA damage

As discussed in chapter 9 (cancer), a 12-week exposure to nicotine-containing vaping product aerosol induced DNA adducts in mouse lung, bladder, and heart tissues (75). It has been proposed that after inhaling nicotine through vaping product aerosol it could be

further metabolised into the highly carcinogenic NNK, which could lead to DNA adducts formation and DNA damage. However, the Lee and others study (75) did not explore whether exposure to nicotine-free vaping aerosol affected DNA adducts in different organs.

In several animal studies that recorded cardiovascular outcomes, exposure to nicotine vaping aerosol was associated with changes in cardiac sympathetic activity and lung function. For example, El-Mahdy and others (76) observed nicotine- and time-dependent elevation in blood pressure with levels similar to that observed after exposure to tobacco cigarette smoke, while Szostak and others (77) demonstrated nicotine-related increase in arterial stiffness parameters (that is, pulse wave velocity and pulse propagation velocity) and isovolumic relaxation time in ApoE -/- mice following vaping product exposure. Furthermore, Espinoza-Derout and others (78) and Hasan and others (79) reported that 3 months of vaping product exposure with nicotine reduced markers of left ventricular function and caused cardiomyocytes ultrastructural abnormalities indicative of cardiomyopathy in ApoE-/- mice on a western diet and a high-fat diet, in comparison with the corresponding nicotine-free vaping product group or saline controls. However, these results were not replicated in the 6-month study by Szostak and others (77) using the same mouse model with a larger sample size per each group. Another study (76) indicated that chronic vaping product exposure in C57BL6 mice resulted in significant changes in left ventricular structure and function coupled with increases in adrenergic vasoconstriction and impairment of vascular endothelial relaxation, with higher nicotine concentrations exerting greater effect.

Several studies have also reported that vaping product-exposed animals were found to have nicotine-related alterations in blood and heart tissue biomarkers linked to oxidative stress and endothelial dysfunction, including increased superoxide generation (76), elevated levels of malondialdehyde, indicating increased reactive oxygen species generation along with mitochondrial DNA damage (78) and increased expression of 4-hydroxynonenal protein adducts (79).

Numerous animal studies have reported vaping product-induced respiratory effects related to nicotine, as described in chapter 10 (respiratory diseases). Briefly, a sub-chronic 4-week but not an acute 3-day exposure to nicotine through inhalation of vaping product aerosol enhanced protein carbonyls and an oxidative stress marker malondialdehyde in bronchoalveolar lavage fluid compared to air-controls, while exposure to nicotine-free vaping aerosol did not (80). Some of the mouse bronchoalveolar lavage fluid cytokines and angiotensin-converting enzyme 2 expression in the lung increased in response to vaping product exposure, with these effects aggravated in the presence of nicotine (81). Consistent with these findings, nicotine-dependent effects on bronchoalveolar lavage fluid inflammatory cell influx and pro-inflammatory mediators have been reported in studies by Wang and others (82, 83). Interestingly, Chapman and others (84) observed substantially different effects of vaping product exposure with and without nicotine in an animal model of

allergic disease, where nicotine-containing vaping products suppressed airway inflammation, independent of flavourings. Furthermore, vaping product exposure with nicotine was associated with significant yet differential changes in the abundance and expression levels of circadian clock genes in mouse lungs (85). In contrast, numerous studies have reported vaping product-induced adverse effects independent of nicotine concentration (81, 86 to 89).

Finally, a considerable body of evidence is available suggesting the effect of nicotine vaping product exposure on body weight. For example, body weight measured over 12 weeks in nicotine-containing vaping product-exposed C57BL/6 mice on a high fat diet was significantly reduced compared to air-controls or nicotine-free vaping product exposed mice (79). A recent study by El-Mahdy and others (76) demonstrated significantly decreased body weight gain in C57BL/6 mice exposed to vaping product aerosols in a dose- and time-dependent manner throughout 60 weeks, as compared to air-controls. Although independent of nicotine concentration, the greatest weight gain inhibition was observed in the nicotine-containing vaping product group, suggesting an important role of nicotine in vaping product-induced changes in body weight. Similarly, 18 weeks of vaping product exposure with and without nicotine in ApoE-/- mice resulted in statistically significantly reduced body weight gain, with the lowest decrease in weight gain found in the absence of nicotine (90). These data are consistent with other studies reporting that exposure to nicotine-containing vaping product caused inhibition in body weight gain in both mice (91 to 93) and rats (94, 95).

On the contrary, no significant difference in body weight was found between animals exposed to vaping product aerosols with various nicotine concentrations and air in other studies (96 to 100). Direct comparison with tobacco cigarettes indicated that the body weight of the nicotine-containing vaping product-exposed animals showed similar profiles to those animals that were exposed to the tobacco cigarette smoke (76, 90, 93, 94), apart from one study finding significant results in the tobacco cigarette group only (97).

Although the mechanism of the vaping product-induced inhibition of weight gain is still unclear, there are several explanations for this effect that may be related to nicotine. Nicotine is known to suppress appetite, while raising basal metabolic rate and lipolysis. This role of nicotine was supported by Shao and others study (91) finding that mice exposed to vaping product aerosol with nicotine had decreased body weight and food intake compared to saline aerosol-treated mice. On the other hand, nicotine stimulates the production of catecholamines, which may delay body weight gain even without affecting food intake. Indeed, Wawryk-Gawda and others (95) demonstrated inhibition of body weight gain in response to vaping product exposure with insignificant variations in food and water intake in rats. However, only a few studies assessed food and water consumption in the exposed animals, and it is yet unclear whether changes in the body weight gain are mediated through insufficient food intake or through other mechanisms. Additionally, further animal studies should consider that repeated restraint could be a

confounding factor in low body weight gain and that a similar experimental setup should be used for control animals.

5.10 Conclusions

In this chapter we discussed the role of nicotine in vaping product use.

As discussed in chapter 3 (vaping among young people) and chapter 4 (vaping among adults), 2021 survey data from England shows that nicotine would appear to play an important driver of adult vaping, but perhaps less so than for tobacco smoking.

Most adults who vape (approximately 87%) use vaping products that contain nicotine. This proportion was about 70% for 11 to 18 year olds, with about half of these saying that their vaping products always contained nicotine, and half sometimes. Among 16 to 19 year olds who reported ever using vaping products with nicotine, approximately 84% said that their products contained nicotine or that some of their products contained nicotine. Overall, the vast majority were using vaping products with less than 20mg/mL nicotine e-liquids and so complied with current vaping product regulations.

Questions on the use of salt-based nicotine products as opposed to freebase nicotine were not frequently included in surveys. Where questions were asked, the responses suggested a considerable amount of uncertainty about whether people who vape were using salt-based vaping products.

Previous reviews indicated that nicotine intake from vaping products was variable and dependent on different product characteristics. The updated evidence presented here also provides conclusive evidence of this variability. The updated evidence from pharmacokinetic studies on vaping show that in general, vaping products provide lower peak nicotine levels and lower overall nicotine levels to users than smoking provides. Also, the pharmacokinetic studies show that exposure to nicotine from vaping varies by product characteristics. The studies suggested that exposure to nicotine tends to increase when:

- using e-liquids with higher nicotine concentration
- using e-liquids based on nicotine salts rather than freebase nicotine
- using tank or modular type vaping devices which provide more exposure than cartridge of disposable models
- people with longer vaping experience vape, as they have more effective puffing behaviour

Time to peak nicotine delivery from vaping products is usually slower compared with smoking a cigarette but varies dependent on the e-liquid nicotine concentration and the type of vaping device. Flavours may also play a role in nicotine delivery and these are reviewed in chapter 6 (flavours).

The pharmacokinetic studies are consistent with the studies discussed in chapter 7 (biomarkers of exposure to nicotine and potential toxicants) which generally showed lower exposure to nicotine when using vaping products over the short term (up to 7 days) compared to smoking. However, there was moderate evidence, in medium to longer term studies (up to 2 years), of similar exposure to nicotine from vaping compared to smoking. For experienced adult vapers, there was substantial evidence of comparable exposure to nicotine from vaping and smoking. There was supportive evidence that over time, people who vape compensate for lower nicotine concentrations by compensatory puffing (such as puffing more frequently, puffing larger volumes of aerosol, or taking longer puffs).

There was substantial evidence from previous reports that the use of vaping products can result in symptoms of nicotine dependency and moderate evidence that the risk and severity of this is lower than for cigarette smoking and would vary by product characteristics. The pharmacokinetic studies reviewed are consistent with this. Our review indicated a plethora of scales that are used to assess nicotine and vaping dependency with as yet, no consensus on which is the optimum scale to assess vaping dependency, making assessment of the risk and severity of vaping dependency in relation to tobacco smoking dependency difficult.

A recent systematic review examining the effects of nicotine concentration and flavours on dependency identified that higher nicotine concentrations might increase abuse potential and appeal of vaping and hence dependency. This could help facilitate complete substitution of tobacco cigarettes. Preliminary evidence also suggested that flavours may interact with nicotine concentrations to affect abuse liability.

The health risks of vaping are reviewed in chapters 8 to 12. Isolating the effects of nicotine on these risks in human studies is complex, partly because only a small minority of people vape non-nicotine products. In general, where studies assessed biomarkers in humans through non-nicotine vaping as well as nicotine vaping, the methodological heterogeneity of the studies limited conclusions. One biomarker, pulse wave velocity did seem to be affected by nicotine in vaping products at least in acute exposure studies. Evidence from the reviewed animal and cell studies suggest some adverse effects of nicotine, but the extent to which these findings can be generalised to humans is currently very unclear.

5.11 Implications

Questions in national surveys sometimes lag behind product developments, such as the use of salt-based vaping products or increasing use of disposable vaping products. Having

an appropriately resourced product surveillance system would help to ensure product developments can be captured by researchers in this field.

Exploring how nicotine labelling could be improved could also be useful as there appears to be an increase in users not knowing how much nicotine was in their vaping products. Additionally, further exploration of the small proportion of adults who use nicotine-free vaping products is warranted, for example assessing duration and frequency of use.

Current evidence indicates that vaping product users with more experience adjust their puffing behaviour to attain higher levels of nicotine. Although this does not compensate for lower overall nicotine exposure after a single vaping session compared with smoking a cigarette, during longer-term or ad libitum vaping sessions experienced vapers reach levels of nicotine comparable to those from smoking (as indicated by nicotine biomarker data). The ability to adjust puffing behaviour when vaping mirrors such behaviour with smoking and suggests that vaping enables users to carefully titrate their nicotine levels. This is of concern when people using vaping products with lower nicotine concentrations compensate by increasing their puffing behaviours as they risk increasing exposure to other potential constituents, an issue explored further in subsequent chapters. There was suggestive evidence from a recent systematic review that limiting nicotine concentrations in vaping products might reduce smoking cessation.

Future research should employ more longitudinal study designs to explore the effect of vaping experience on vapers' puffing behaviour, nicotine intake and dependency over time. This is important for people who have smoked as well as never smokers. For never smokers who initiate nicotine use through vaping, measurements are needed across a range of vaping products and their characteristics to assess whether higher nicotine limits (>20mg/mL) impact dependency of vaping behaviours and how these might interact with protonation, flavours and other characteristics. Research on longer-term vaping behaviour would also allow clarification of how the use of different nicotine strength e-liquids over time is associated with dependency and potential health risks.

Having a global consensus for assessing nicotine and product dependency would facilitate the measurement of these important attributes between vaping and smoking, across different vaping products, and with different groups of users (such as adults and youth). In England, it is important for researchers to keep abreast of the ongoing research in this area.

Agreeing a standard protocol for vaping product pharmacokinetic studies would also enable meaningful comparisons across different vaping products and e-liquid characteristics. However, more long-term ad libitum pharmacokinetic studies are also needed to reflect how users' experience and idiosyncratic puffing behaviours affect nicotine delivery and dependency.

Isolating the risks of nicotine to health from the risks of other vaping constituents is difficult in human studies compared to animal and cell studies. Having standards, particularly for human cell research, may strengthen how widely or generally applicable such studies are to vapers. Such standards would also be beneficial in helping to examine the impact of nicotine.

Table 1. Commonly used measures for assessing nicotine, tobacco and vaping product dependency

Scale or measure	Details	Selected key references
Fagerstrom Test for Nicotine Dependence, changed to Fagerstrom Test for Cigarette Dependence (FTND/FTCD). Has been adapted for vaping (e-FTCD).	6 items, weighted	Heatherton et al., 1991 (101); Fagerstrom, 2012 (22)
Time to First Cigarette/e-cigarette (TTFU)	1 item from FTND	Muscat et al., 2009 (102) Fagerstrom, 2003 (103)
Heaviness of Smoking Index (HSI) Also, adapted to Heaviness of Vaping Index (HVI)	2 items from FTND (TTFU and cigarettes per day)	Etter et al., 1999 (104) Liu et al., 2017 (105)
Mood and Physical Symptom Scale (MPSS) scores	7 variables in relation to past 24 hours (e.g depressed) and frequency and strength of urges to smoke; severity of 3 further symptoms (e.g., constipation)	West & Hajek, 2004 (106)
Penn State Electronic Cigarette Dependence Index (PSECDI)	10 items, weighted; can be compared with the Penn State Cigarette Dependence Index, which was adapted from FTND, HONC, and other withdrawal, craving and urges to use measures.	Foulds et al., 2015 (107)

Scale or measure	Details	Selected key references
Brief-Wisconsin Inventory of Smoking Dependence Motives (WISDM); Wisconsin Inventory of Smoking Dependence Motives Has been adapted for vaping (e-WISDM)	37 items, with 11 subscales 68 items, assessing 13 factors	Smith et al., 2010 (108)
Questionnaire on Smoking Urges (QSU)	32 items, measuring 2 dimensions of craving	Tiffany & Drobes, 1991 (109)
Hooked on Nicotine Checklist (HONC)	10 items, measuring loss of autonomy – sensitive to detecting dependence at low levels	DiFranza et al., 2002 (110)
Cigarette Dependence Scale*	12 items	Etter et al., 2003 (111)
Nicotine Dependence Syndrome Scale (NDSS)	19 items measuring 5 dimensions of dependence	Shiffman et al., 2004 (112)
Minnesota Nicotine Withdrawal Scale (MNWS)	8 items, based on DSM-IV	Toll et al., 2007 (113)
Product Evaluation Scale (PES) Or, individual items e.g. Craving item of the modified cigarette evaluation questionnaire (mCEQ)	Up to 21 items, including satisfaction (4 items), psychological reward (4 items), aversion (4 items), relief (5 items) but there can be other combinations	PES is adapted from the mCEQ Cappelleri et al., 2007 (114); Hatsukami et al, 2013 (115)

Scale or measure	Details	Selected key references
Dimensions of Tobacco Dependence Scale (DTDS)	54 items, 4 factors assessing tobacco dependence in adolescence: social/emotional/sensory/physical reinforcement	Johnson et al., 2005 (116)
Patient-reported Outcomes Measurement Information Systems (PROMIS)	32 items	Shadel et al., 2014 (117); Edelen et al., 2012 (118)
E-cigarette Dependence Scale (EDS)	Adapted from PROMIS	Morean et al., 2019, 2020 (59, 119)
Tobacco Craving Questionnaire*	17 items assessing 4 constructs: emotionality, expectancy, compulsivity, purposefulness	Heishman et al., 2003 (120)
Population Assessment of Tobacco and Health dependency symptoms (PATH) study*)	20 items [but see Tobacco Dependence Index which is drawn from these PATH items]	National Institute of Health. National longitudinal study of tobacco use. 2017 (121, 122).
Tobacco Dependence Index	16 items, drawn from Wisconsin Inventory of Smoking Dependence Motives (WISDM), NDSS, & DSM (1 time), validated for smoking; subsequently validated for vaping	Strong et al., 2017 (60) Strong et al., 2020 (123)
Questionnaire of Vaping Craving (QVC)	10 items	Dowd et al., 2019 (124)

Scale or measure	Details	Selected key references
Other	Strength of urges to smoke Glover Nilsson Smoking Behavioral Questionnaire E-Cigarette Addiction Severity Index (EASI) Cigarette Withdrawal Scale Profile of Mood State Manual Shiffman Jarvik Withdrawal Scale Smoker Complaints Scale Wisconsin Smoking Withdrawal Scale	Fidler et al., 2011 (125) Bover et al., 2008 (126) Glover et al., 2005 (127) Vogel et al., 2020 (64)

Notes: * Adapted from Bold et al. (58). DSM—Diagnostic and Statistical Manual of Mental Disorders; DTDS— Dimensions of Tobacco Dependence Scale; EDS— E-cigarette Dependence Scale; FTCD—Fagerstrom Test for Cigarette Dependence; FTND—Fagerstrom Test for Nicotine Dependence; HONS—Hooked on Nicotine Checklist; HSI—Heaviness of Smoking Index; mCEQ—modified cigarette evaluation questionnaire; MPSS—Mood and Physical Symptom Scale; NDSS—Nicotine Dependence Syndrome Scale; PATH—Population Assessment of Tobacco and Health; PSECDI – Penn State Electronic Cigarette Dependence Index; PES—Product Evaluation Scale; PROMIS—Patient-reported Outcomes Measurement Information Systems; TTFU—Time to First Cigarette/e-cigarette; WISDM—Wisconsin Inventory of Smoking Dependence Motives.

Table 2. Summary of studies exploring pharmacokinetic nicotine delivery profile of vaping products (VP)

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
Cross-over				
Ebajemito et al., 2020, UK (53)	n = 24 Dual users: healthy, smoking ≥1 year, maximum of 21 TC per week, not planning to quit, urinary cotinine ≥200ng/mL. Mean (SD) age: 37.3 (12.6), 37.5% females, mean (SD) BMI: 24.8 (3.5).	1. Vaping, ePen, 18mg/mL unprotonated (n=22): ad lib use of cartridge VP (Vype ePen) with blended tobacco flavoured 18mg/mL unprotonated nicotine for 5 minutes. 2. Vaping, ePen3, 18mg/mL unprotonated (n=23): ad lib use of cartridge VP (Vype ePen3) with blended tobacco flavoured, 18mg/mL unprotonated nicotine for 5 minutes. 3. Vaping, ePen3, 18mg/mL medium protonation (n=23): ad lib use of cartridge VP (Vype ePen3) with MasterBlend tobacco flavoured, 18mg/mL medium protonation nicotine for 5 minutes. 4. Vaping, ePen3, 30mg/mL high protonation (n=23): ad lib use of cartridge VP (Vype ePen3) with MasterBlend	**Maximum plasma nicotine concentration (C_{max})** 1. Mean (SD)= 5.82 (3.81)ng/mL. 2. 8.01 (5.38). 3. 12.5 (6.81) 4. 16.8 (9.24). 5. 9.79 (8.4). 6. 7.33 (4.56). 7. 18.5 (12.5). 8. 16.3 (10.4). **Time to maximum nicotine concentration (T_{max})** 1. Mean (SD) = 12.2 (10.3) minutes. 2. 10.7 (9.34).	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
		tobacco flavoured, 30mg/mL high protonation nicotine for 5 minutes. 5. Vaping, ePen3, 18mg/mL medium protonation (n=22): 10 puffs, one every 30 seconds of cartridge VP (Vype ePen3) with MasterBlend tobacco flavoured, 18mg/mL medium protonation nicotine. 6. Vaping, ePen3, 12mg/mL low protonation (n=23): ad lib use of cartridge VP (Vype ePen3) with MasterBlend tobacco flavoured, 12mg/mL low protonation nicotine for 5 minutes. 7. Smoking, ad lib (n=23): ad lib smoking a TC (B&H Skyblue) in 5 minutes. 8. Smoking, controlled (n=23): 10 puffs, one every 30 seconds of the same TC.	3. 6.04 (2.33). 4. 5.7 (2.53). 5. 6.64 (2.59). 6. 7.83 (5.4). 7. 7.22 (5.67) 8. 7 (2.34). **Area under the concentration–time curve ($AUC_{0\text{-}120}$)** 1. Mean (SD) = 298 (137) ng/mL*h 2. 365 (169) 3. 478 (222). 4. 628 (294). 5. 350 (199). 6. 317 (149).	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			7. 717 (241). 8. 666 (212).	
Goldenson et al., 2020, US (54)	n = 66 Smokers: current smoking of ≥10 TC for the last ≥12 months, urinary cotinine ≥500 ng/mL, eCO >10 ppm. Mean (SD) age: 41.1 (10.8), 50% females, 63.6% white, 27.3% African American, 6.1% Hispanic, 3% of other ethnicity.	1. Vaping, Virginia tobacco (n=63-65): 10 3-second puffs of a pod VP (JUUL) with Virginia tobacco flavour and 59mg/mL nicotine salt. 2. Vaping, Mango (n=63-65): 10 3-second puffs of a pod VP (JUUL) with Mango flavour and 59mg/mL nicotine salt. 3. Vaping, Mint (n=63-65): 10 3-second puffs of a pod VP (JUUL) with Mint flavour and 59mg/mL nicotine salt. 4. Vaping, Creme (n=63-65): 10 3-second puffs of a pod VP (JUUL) with Creme flavour and 59mg/mL nicotine salt. 5. Smoking (n=63-65): 10 3-second puffs of own brand TC. 6. Vaping, Vuse (n=63-65): 10 3-second puffs of a cartridge VP (Vuse) with	**Maximum plasma nicotine concentration (C_{max})** Stat. sig. lower for all vaping products and nicotine gum compared with TC (15.4 ng/mL). NS diff. between pod VP (6.6-8.6 ng/mL), cartridge VP (6.8 ng/mL) and nicotine gum (5.6 ng/mL). **Time to maximum nicotine concentration (T_{max})** NS diff. between pod VP and TC (ps=0.26-0.94). NS diff. between pod VP and cartridge VP (ps>0.08). Stat. sig. faster in pod VP compared with nicotine gum (ps<0.001). **Area under the concentration–time curve (AUC_{0-60})** Stat. sig. lower for all vaping products and nicotine gum compared with TC. Stat. sig. higher in pod VP (Virginia Tobacco, Mango and Mint) compared with cartridge VP or nicotine gum.	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
		tobacco flavour and 48mg/mL nicotine e-liquid. 7. Nicotine gum (n=63-65): 'chew and park' method use for 30 minutes.	Stat. sig. higher in pod VP (Virginia Tobacco, Mango and Mint) compared with pod VP Crème flavour. **Rate of plasma nicotine rise** Stat. sig. lower for all vaping products and nicotine gum compared with TC (ps<0.004). NS diff. between pod VP and cartridge VP (ps>0.19). Stat. sig. higher in pod VP compared with nicotine gum (ps<0.02).	
Goldenson et al., 2021, US (35)	n = 25 Smokers: current smoking of ≥10 non-menthol TC for the last ≥12 months, urinary cotinine ≥200 ng/mL, eCO >10 ppm. Mean (SD) age: 41.5 (9.9), 20% females, all of the white ethnicity.	1. Vaping, 59mg/mL (n=25): 10 3-second puffs of a pod VP (JUUL, silica wick) with Virginia Tobacco flavour and 59mg/mL nicotine salt. 2. Vaping, 59mg/mL (n=25): ad libitum use of the same VP for 5 minutes. 3. Vaping, silica wick, 18mg/mL (n=25): 10 3-second puffs of a pod VP (JUUL, silica wick) with Golden Tobacco flavour and 18mg/mL nicotine salt.	**Maximum plasma nicotine concentration (C_{max})** 1. Geometric mean (SD) = 9.3 (1.7) ng/mL 2. 8.3 (1.4) 3. 3.2 (1.8) 4. 3.5 (1.5) 5. 3.3 (1.6) 6. 3.3 (1.7)	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
		4. Vaping, silica wick, 18mg/mL (n=25): ad libitum use of the same VP for 5 minutes. 5. Vaping, cotton wick, 18mg/mL (n=25): 10 3-second puffs of a pod VP (JUUL, cotton wick) with Golden Tobacco flavour and 18mg/mL nicotine salt. 6. Vaping, cotton wick, 18mg/mL (n=25): ad libitum use of the same VP for 5 minutes. 7. Vaping, cotton wick, 9mg/mL (n=25): 10 3-second puffs of a pod VP (JUUL, cotton wick) with Golden Tobacco flavour and 9mg/mL nicotine salt. 8. Vaping, cotton wick, 9mg/mL (n=25): ad libitum use of the same VP for 5 minutes. 9. Smoking (n=25): 10 3-second puffs of own-brand TC.	7. 2.1 (1.6) 8. 2.3 (1.6) 9. 15.7 (1.6) 10. 18.4 (1.7) **Time to maximum nicotine concentration (T_{max})** 1. Mean (SD) = 6.2 (2.4) minutes. 2. 6.4 (2) 3. 6.3 (1.6) 4. 6.5 (2.2) 5. 5.8 (1.8) 6. 7.1 (3.2) 7. 6.6 (5.4) 8. 6.7 (2.4)	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
		10. Smoking (n=25): ad lib smoking own-brand TC.	9. 7.8 (5) 10. 6.7 (1.7) **Area under the concentration–time curve (AUC_{0-60})** 1. Geometric mean (SD) = 5 (1.4) h*ng/mL. 2. 4.6 (1.4) 3. 1.7 (1.6) 4. 1.8 (1.5) 5. 1.8 (1.4) 6. 2.1 (1.4) 7. 1.2 (1.3) 8. 1.2 (1.4) 9. 8.5 (1.4) 10. 9.2 (1.4)	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
Hiler et al., 2017, US (36)	n = 64 Dual users (n=33): self-reported VP users for ≥3 months, using ≥1 mL of e-liquid daily with 8mg/mL nicotine, smoking ≥5 TC daily and with ≥10 ppm eCO at baseline. Mean (SD) age: 30.3 (8.4), 18.2% females, 72.7% Caucasian. Smokers (n=31): VP-naïve, self-reported smoking ≥10 TC per day, <5 VP uses in their lifetime and with ≥15 ppm	Four cross-over conditions separated by >48 hours: 1. Vaping, 0mg/mL (n=64): two vaping bouts 60 minutes apart consisting of 10 puffs every 30 seconds on a tank-type VP (eGo, 3.3 volt, 1000 mAh battery, 1.5 Ω dual coil) with 70%/30% PG/VG tobacco or menthol flavoured (chosen by participants), using 0mg/mL nicotine e-liquid. 2. Vaping, 8mg/mL (n=64): same procedure using 8mg/mL e-liquid. 3. Vaping, 18mg/mL (n=64): same procedure using 18mg/mL e-liquid. 4. Vaping, 36mg/mL (n=64): same procedure using 36mg/mL e-liquid.	**Plasma nicotine concentration** Dual users: NS diff. in 0mg/mL nicotine condition compared with baseline. Stat. sig. increase in 8, 18 and 36mg/mL nicotine conditions compared with baseline ($p<0.05$). Smokers: NS diff. in 0 and 8mg/mL nicotine conditions compared with baseline. Stat. sig. increase in 18 and 36mg/mL nicotine conditions compared with baseline ($p<0.05$). Between-group: NS diff. in 0mg/mL nicotine conditions. Stat. sig. higher in dual users compared with smokers in 8, 18 and 36mg/mL nicotine conditions ($p<0.05$).	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
	eCO at baseline. Mean (SD) age: 30.9 (9.9), 41.9% females, 51.6% Caucasian.			
Hiler et al., 2020, US (37)	n = 32 Vapers: self-reported smoking <5 TC per day and using other tobacco products <4 times per week. Use >1 mL e-liquid daily, use ≥3mg/mL nicotine e-liquid, had been using a VP ≥3 months. Mean (SD) age: 25.6 (7.1), 25% females, 59.4% Caucasian.	Four 3.5-hour cross-over sessions separated by ≥48 hours 1. Vaping, 40.5 W, 0.5 Ω, 3mg/mL nicotine (n=32): 10 puffs every 30 seconds and 60 minutes ad lib use separated by 60 minutes of a modular VP (Kangertech Subtank) with 30%/70% PG/VG, pear flavoured and 3mg/mL nicotine strength e-liquid. 2. Vaping, 40.5 W power, 0.5 Ω, 8mg/mL nicotine (n=32). 3. Vaping, 13.5 W power, 1.5 Ω, 3mg/mL nicotine (n=32). 4. Vaping, 13.5 W power, 1.5 Ω, 8mg/mL nicotine (n=32).	**Plasma nicotine concentration** 1. 40.5 W, 0.5 Ω, 3mg/mL nicotine: stat. sig. higher with ad libitum > controlled > baseline. 2. 40.5 W, 0.5 Ω, 8mg/mL nicotine: stat. sig. higher with ad libitum > controlled > baseline. 3. 13.5 W power, 1.5 Ω, 3mg/mL nicotine: NS diff. after controlled compared with baseline. Stat. sig. increase after ad lib use compared with baseline 4. 13.5 W power, 1.5 Ω, 8mg/mL nicotine: stat. sig. higher with ad libitum > controlled > baseline. Between-group: stat. sig. higher	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			increase following controlled use in 0.5 Ω, 8mg/mL condition compared with other conditions ($p<0.05$). Stat. sig. higher increase after ad lib use in 40.5 W, 0.5 Ω, 8mg/mL condition compared with 1.5 Ω, 3mg/mL and 1.5 Ω, 8mg/mL conditions ($p<0.05$). NS diff. after ad lib use between 0.5 Ω, 8mg/mL and 0.5 Ω, 3mg/mL nicotine conditions ($p>0.05$). Stat. sig. higher in both 0.5 Ω, 40.5 W conditions compared with both 1.5 Ω, 13.5 W conditions.	
Mallock et al., 2021, Germany (38)	n = 32 Vapers (n=17): daily VP use for >3 months, no daily smoking for >3 months. Smokers (n=15): daily smoking of >10 TC for >5 years.	1. Vaping, 'initial' (n=11): 3-second puffs every 30 seconds for 5 minutes of the 'initial' pod VP version (JUUL) with rich tobacco flavour and 18mg/mL nicotine salts. 2. Vaping, 'modified' (n=13): 3-second puffs every 30 seconds for 5 minutes of the 'modified' pod VP version (JUUL) with	**Maximum plasma nicotine concentration (C_{max})** 1. Vaping, 'initial' version (n=11): Geometric mean (%CV) = 6.5 (79) ng/mL. 2. Vaping, 'modified' version (n=13): 6.3 (69) ng/mL. 3. Smoking: 13.1 (77). **Time to maximum nicotine**	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
	Median (IQR) age: 28 (25-33), 40.6% females.	rich tobacco flavour and 18mg/mL nicotine salts. 3. Smoking (n=15): 3-second puffs every 30 seconds for 5 minutes smoking a TC (Marlboro Red).	**concentration (T_{max})** 1. Vaping, 'initial' version (n=11): median (range) = 4 (2-6) minutes. 2. Vaping, 'modified' version (n=13): 6 (2-8). 3. Smoking: 8 (6-30). **Area under the concentration–time curve (AUC_{0-30})** 1. Vaping, 'initial' version (n=11): Geometric mean (%CV) = 110.9 (49) ng/mL*min. 2. Vaping, 'modified' version (n=13): Geometric mean (%CV) = 103.3 (63) ng/mL*min. 3. Smoking: 257 (49).	
O'Connell et al., 2019, US (39)	n = 15 Smokers: smoked ≥10 TC per day for ≥1 last year. Verified by	1. Vaping, 25mg/mL freebase (n=15): 10 3-second puffs every 30 seconds of a pod VP (myblu, 350 mAh battery, 1.3 Ω) with	**Maximum plasma nicotine concentration (C_{max})** 1. 25mg/mL freebase: geometric mean (%CV)=5.0 (49.9) ng/mL; stat.	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
	eCO >10 ppm and urinary cotinine ≥500 ng/mL. Mean (SD) age: 42.3 (12.4), 40% females, mean (SD) BMI: 28.1 (5.1).	tobacco-flavoured 25mg/mL freebase nicotine e-liquid. 2. Vaping, 16mg/mL salt (n=15): the same pod VP with tobacco-flavoured 16mg/mL salt nicotine. 3. Vaping, 25mg/mL salt (n=15): the same pod VP with tobacco-flavoured 25mg/mL salt nicotine. 4. Vaping, 40mg/mL salt (n=15): the same pod VP with tobacco-flavoured 40mg/mL salt nicotine. 5. Vaping, 48mg/mL salt (n=15): 10 3-second puffs every 30 seconds of a tank VP (blu PRO, 1100 mAh, 1.8 Ω) with tobacco-flavoured 48mg/mL salt nicotine. 6. Smoking (n=15): a single TC with puffs taken every 30 seconds.	sig. lower compared with TC ($p<0.001$). 2. 16mg/mL salt: 6.51 (76.5); stat. sig. lower compared with TC ($p<0.001$). 3. 25mg/mL salt: 7.58 (80.6); stat. sig. lower compared with TC ($p<0.01$). 4. 40mg/mL salt (pod VP): 10.3 (83.6); NS diff. compared with TC. 5. 48mg/mL salt (tank VP): 4.9 (108.3); stat. sig. lower compared with TC ($p<0.001$). Stat. sig. lower compared with 40mg/mL salt (pod VP, ($p<0.05$). 6. TC: 17.8 (49.6) ng/mL. **Time to maximum nicotine concentration (T_{max})** NS diff. between groups. 1. 25mg/mL freebase: median (range)=8.0 (2.3-15.1) minutes.	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			2. 16mg/mL salt: 7 (4.0-15.1). 3. 25mg/mL salt: 6.0 (4.6-16.8). 4. 40mg/mL salt (pod VP): 7.9 (2-15). 5. 48mg/mL salt (tank VP): 6.9 (2.4-15). 6. TC: 8.1 (5-15.1). **Area under the concentration–time curve (AUC_{0-30})** 1. 25mg/mL freebase: geometric mean (CV%)=99 (35.8) ng*min/mL; stat. sig lower compared with TC ($p<0.001$). 2. 16mg/mL salt: 118.5 (60.8); stat. sig lower compared with TC ($p<0.001$). 3. 25mg/mL salt: 125.2 (53.4); stat. sig lower compared with TC ($p<0.001$). 4. 40mg/mL salt (pod VP): 190.7 (71.8); NS diff compared with TC.	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			5. 48mg/mL salt (tank VP): 84.8 (89.8); stat. sig lower compared with TC (p<0.001). 6. TC: 324.9 (35.8) ng*min/mL.	
Phillips-Waller et al., 2021, UK (40)	n = 22 Dual users: VP users who were also occasionally smoking. Mean age: 31, 18% females.	1. Vaping, pod (n=22): ad lib use for 5 minutes of a pod VP (JUUL) with Virginia Tobacco flavour and 59mg/mL nicotine salts. 2. Vaping, tank (n=8): ad lib use for 5 minutes of a tank (KangerTech EVOD) or mod VP(Innokin iTaste MVP 2, 4.8 V) with tobacco flavour and 20mg/mL nicotine e-liquid. 3. Smoking (n=22): ad lib smoking own TC in 5 minutes. 4. Other (n=22): ad lib use of a single tobacco-flavoured HTP in 5 minutes.	**Maximum plasma nicotine concentration (C_{max})** 1. Median (IQR)=19.6 (8.9-36.3) ng/mL; stat. sig. higher compared with HTP (p=0.008). 2. 12.6 (7.1-13.9); NS diff. compared with HTP (p=0.093). 3. 12.9 (7.2-28.6); NS diff. compared with HTP (p=0.095). 4. 8.3 (4.5-19.3). **Time to maximum nicotine concentration (T_{max})** 1. Median (IQR)=4 (2-6) minutes; NS diff. compared with HTP.	High

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			2. 7.5 (5.3-14.1); stat. sig. slower compared with HTP (p=0.018). 3. 6 (4-8); stat. sig. slower compared with HTP (p=0.031). 4. 4 (4-6). **Area under the concentration–time curve (AUC_{0-30})** 1. Median (IQR)= 343.2 (168.1-461.1); stat. sig. higher compared with HTP (p=0.002). 2. 199.3 (114.1-263.1); NS diff. compared with HTP. 3. 314.7 (136.4–465.6); stat. sig. higher compared with HTP (p=0.006). 4. 152.0 (91.2–254.5).	
Phillips-Waller et al., 2021, UK (41)	n = 18 Dual users: daily VP users who smoked TC	1. Vaping, EU (n=18): ad lib use for 5 minutes of a pod VP (JUUL) with Golden	**Maximum plasma nicotine concentration (C_{max})** 1. Median (IQR)= 3.8 (2.5-7.5) ng/mL.	High

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
	occasionally. Median (IQR) age: 29.5 (25.8-41.0), 11.1% females.	Tobacco flavour and 20mg/mL nicotine salts. 2. Vaping, US (n=18): ad lib use for 5 minutes of a pod VP (JUUL) with Virginia Tobacco flavour and 59mg/mL nicotine salts. 3. Smoking (n=18): ad lib smoking own-brand TC in 5 minutes. 4. Vaping, other (n=7): ad lib use for 5 minutes of different VPs with tobacco flavour and 16-48mg/mL nicotine e-liquids. Findings NR.	2. 21.1 (9.9-36.3); stat. sig. higher compared with Vaping, EU (p<0.001). 3. 12.9 (8-35.6); stat. sig. higher compared with Vaping, EU (p<0.001). **Time to maximum nicotine concentration (T_{max})** 1. Median (IQR)=6 (4-8) minutes. 2. 4 (2-6); NS diff. compared with Vaping, EU (p=0.068). 3. 5 (4-8); NS diff. compared with Vaping, EU (p=0.605). **Area under the concentration–time curve (AUC_{0-30}, n=14)** 1. Mean (SD)= 77.3 (31). 2. 355.9 (173.7); stat. sig. higher compared with Vaping, EU (p<0.001). 3. 324.8 (208.9); stat. sig. higher compared with Vaping, EU (p<0.001).	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
Ruther et al., 2018, Germany (42)	n = 20 Vapers (n=9): vaping ≥3 months and had not smoked a TC for the past month. Mean (SD) age: 28.5 (8.9). Smokers (n=11): ≥5 TC per day for the past 3 years. Mean (SD) age: 26.2 (6.9).	1. Vaping, cartridge VP (n=9, vapers): 10 4-seconds puffs with 26 seconds inter-puff intervals of a cartridge VP (American heritage, Vype or Blu) with strawberry/mint flavoured, 18mg/mL nicotine strength e-liquid. 2. Vaping, tank VP (n=9, vapers): same puffing regime of a tank VP (Aspire/Joytech eGo C2, 650 mAh battery, 1.8 Ω) with strawberry/mint flavoured, 18mg/mL nicotine strength e-liquid. 3. Smoking (n=11, smokers): 10 2-seconds puffs with 28 seconds inter-puff intervals of a Marlboro Red TC (0.8 mg nicotine).	**Plasma nicotine concentration** 1. Vaping, cartridge VP (5 minutes): mean (SD)=5.5 (3.2) ng/mL. NS diff. compared with tank VP (p=0.205). Stat. sig. lower compared with TC (p<0.001). 2. Vaping, tank VP (5 minutes): 9.3 (7.9). Stat. sig. lower compared with TC (p=0.016). 3. TC (5 minutes): 17.1 (8.1) ng/mL.	High
Spindle et al., 2018, US (43)	n = 30 Vapers/dual users: healthy, smoking <5 TC per day, using ≥1ml of e-liquid per day and	Vaping (n=30): two monitored sessions separated by 60 minutes using tank VP (eGo 3.3V battery with 1.5 ohm, dual-coil, 510 cartomizer, 7.3W) with 18mg/mL nicotine of tobacco flavour for 10 puffs every 30 seconds. PG/VG ratios differed:	**Area under the concentration–time curve (AUC_{0-55})** 100% PG: mean (SD)= 276.75 (221.49) ng*min/mL. 2%/98%: 178.3 (183.8); stat. sig. lower compared with 100% PG (p<0.05).	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
	using a VP with ≥6mg/ml nicotine for ≥3 months. Mean (SD) age: 26.9 (7.1), 3.3% females, 70% Caucasian, 13.3% Asian, 6.7% African American, 10% of other ethnicity, mean (SD) CPD: 0.03 (0.2).	1. 100% PG 2. 55%/45% (results NR) 3. 20%/80% 4. 2%/98%	**Area under the concentration–time curve (AUC_{60-105})** 100% PG: mean (SD)= 373.2 (274.1) ng*min/mL. 20%/80%: 251.9 (224.5); stat. sig. lower compared with 100% PG ($p<0.05$). 2%/98%: 257.8 (217.3); stat. sig. lower compared with 100% PG ($p<0.05$).	
St. Helen et al., 2020, US (44)	n = 36 Dual users: smoking ≥5 TC per day over the past 30 days and use the same VP ≥1 per day daily on 15 of the past 30 days. Mean (SD) age: 35.4	1. Vaping (n=36): 15 puffs (cartridge and pod users, n=15) or 10 puffs (tank and mod users, n=21), each puff every 30 seconds of own VP with varied flavours and nicotine e-liquid strengths. Number of puffs was selected to deliver similar nicotine levels as that of a TC (~1 mg). 2. Smoking (n=36): smoking own TC to completion.	**Maximum plasma nicotine concentration (C_{max})** 1. Vaping: mean (SD) 6.1 (5.5) ng/mL. 2. Smoking: 20.2 (11.1); stat. sig. higher compared with vaping ($p<0.001$). **Plasma nicotine concentration** 1. Vaping: mean (SD)=0.9 (0.7) mg.	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
	(11.7), 22.2%.		2. Smoking: 2.2 (1.2); stat. sig. higher compared with vaping (p<0.001). **Time to maximum nicotine concentration (T_{max})** 1. Vaping: mean (SD) 6.5 (5.4) minutes. 2. Smoking: 2.7 (2.4); stat. sig. faster compared with vaping (p<0.001). **Area under the concentration–time curve (AUC_{0-240})** 1. Vaping: mean (SD) 550 (438) ng/mL*min.. 2. Smoking: 1368 (665); stat. sig. higher compared with vaping (p<0.001). **Terminal elimination half-life ($t_{1/2}$)** 1. Vaping: mean (SD)=137.6 (39.3) minutes.	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			2. Smoking: 121.2 (33.9); stat. sig. shorter compared with vaping (p=0.021).	
Stiles et al., 2018, US (45)	n = 71 Smokers: self-reported smoking ≥10 menthol TC per day for ≥6 months, smoking their first TC within 30 minutes of waking up. Mean (SD) age: 34.3 (10.1), 38.0% females, mean (SD) BMI: 29 (4.8).	1. Vaping, 14mg/mL (n=71): up to 10 minutes ad lib use of a cartridge VP (Vuse Solo) with menthol flavour and 14mg/mL nicotine e-liquid. 2. Vaping, 29mg/mL (n=71): up to 10 minutes ad lib use of a cartridge VP (Vuse Solo) with menthol flavour and 29mg/mL nicotine e-liquid. 3. Vaping, 36mg/mL (n=71): up to 10 minutes ad lib use of a cartridge VP (Vuse Solo) with menthol flavour and 36mg/mL nicotine e-liquid. 4. Smoking (n=71): smoking own TC. 5. Other (n=71): up to 30 minute use of nicotine gum (Nicorette White Ice Mint,	**Maximum plasma nicotine concentration (C_{max})** 1. Geometric LS mean (95% CI) = 2.45 (2.08-2.9) ng/mL; stat. sig. lower compared with smoking and nicotine gum conditions (ps<0.05). 2. 3.4 (2.87-4.02); stat. sig. lower compared with smoking condition (p<0.05). 3. 3.94 (3.32-4.67); stat. sig. lower compared with smoking condition (p<0.05). 4. 18.04 (15.2-21.41). 5. 4.8 (4.06-5.69). **Time to maximum nicotine concentration (T_{max})**	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
		4 mg nicotine) by 'park and chew' method.	1. Median (95% CI) = 19.89 (15.35-29.93) minutes; stat. sig. slower compared with smoking (p<0.05). Stat. sig. faster compared with nicotine gum (p<0.05). 2. 15.1 (14.89-19.97); stat. sig. slower compared with smoking (p<0.05). Stat. sig. faster compared with nicotine gum (p<0.05). 3. 10.13 (9.97-14.92); stat. sig. slower compared with smoking (p<0.05). Stat. sig. faster compared with nicotine gum (p<0.05). 4. 7.43 (6.95-7.52) 5. 45.04 (44.96-46.54). **Area under the concentration–time curve (AUC_{0-360})** 1. Geometric LS mean (95% CI) = 412.34 (358.31-474.52) ng*min/mL; stat. sig. lower compared with	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			smoking and nicotine gum conditions (ps<0.05). 2. 545.14 (473.14-628.1); stat. sig. lower compared with smoking and nicotine gum conditions (ps<0.05). 3. 516.15 (447.11-595.86); stat. sig. lower compared with smoking and nicotine gum conditions (ps<0.05). 4. 1556.44 (1347.9-1797.23). 5. 844.01 (732.23-972.92).	
Voos et al., 2019, US (46)	n = 18 Smokers: smoked ≥ 10 TC per day, verified eCO≥8ppm, FTND> 4. Mean (SD) age: 41.3 (9.7), 50% females.	1. Vaping, disposable (n=18): 20 puffs in total, 30 seconds between puffs, of a disposable VP (v2, 2.9 Ω, 3.96 V) with 18mg/mL nicotine e-liquid. 2. Vaping, cartridge (n=18): same puffing of a cartridge VP (Green Smoke, 3.4 Ω, 3.8 V) with 24mg/mL nicotine e-liquid.	**Maximum plasma nicotine concentration (C_{max})** All stat. sig. lower compared with own-brand TC. 1. Vaping, disposable: median (range)= 4.07 (0.08–16.5) ng/mL. 2. Vaping, cartridge: 4.16 (0.71–16.2). 3. Vaping, tank: 5.52 (0.16–23.0).	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
		3. Vaping, tank (n=18): same puffing of a tank type VP (eGO, v2, 3.3 Ω, 4.14 V) with 24mg/mL nicotine e-liquid. 4. Vaping, modular (n=18): same puffing of a modular VP (iTazte, VTR vaporizer, 2.6 Ω, 6.1 V) with 24mg/mL nicotine e-liquid. 5. Other, e-Cigar (n=18): same puffing of an e-Cigar (Cuvana) with 18mg/mL nicotine e-liquid. 6. Other, e-Pipe (n=18): same puffing of an e-Pipe (Smoktech, 2.5 Ω, 6.0 V) with 24mg/mL nicotine e-liquid. 7. Smoking (n=18): at libitum smoking own-brand TC.	4. Vaping, modular: 6.60 (0.06–39.5). 5. Other, e-Cigar: 3.21 (0.06–18.6). 6. Other, e-Pipe: 5.31 (0.82–33.6). 7. Smoking: 18.9 (3.41–74.4) ng/mL. **Time to maximum nicotine concentration (T_{max})** 1. Vaping, disposable: median (range)= 13 (2–45) minutes; stat. sig. longer compared with TC. 2. Vaping, cartridge: 10 (2-120); stat. sig. longer compared with TC. 3. Vaping, tank: 10 (4-45); NS diff. compared with TC. 4. Vaping, modular: 10 (2-120); stat. sig. longer compared with TC. 5. Other, e-Cigar: 10 (5-120); NS diff. compared with TC.	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			6. Other, e-Pipe: 10 (4-45); stat. sig. longer compared with TC. 7. Smoking: 5.5 (2-30) minutes. **Area under the concentration–time curve (AUC_{0-10})** All stat. sig. lower compared with own-brand TC. 1. Vaping, disposable: median (range)= 16.3 (0.20–79.0) ng/mL/min. 2. Vaping, cartridge: 18.1 (4.40–92.9). 3. Vaping, tank: 18.9 (0.20–106.6). 4. Vaping, modular: 47.3 (0.20–218.8). 5. Other, e-Cigar: 13.1 (0.20–85.4). 6. Other, e-Pipe: 20.2 (2.01–192.5). 7. Smoking: 126.3 (3.59–396.0) ng/mL/min. **Area under the concentration–time curve (AUC_{0-120})**	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			1. Vaping, disposable: median (range)= 88.6 (0.78–334.6) ng/mL/min; stat. sig. lower compared with TC. 2. Vaping, cartridge: 121.9 (6.96–509.8); stat. sig. lower compared with TC. 3. Vaping, tank: 232.8 (1.48–920.9); NS diff. compared with TC. 4. Vaping, modular: 272.3 (0.44–1271); NS diff. compared with TC. 5. Other, e-Cigar: 55.9 (0.44–616.8); stat. sig. lower compared with TC. 6. Other, e-Pipe: 113.7 (7.49–1179); NS diff. compared with TC. 7. Smoking: 347.5 (17.1–2354) ng/mL/min.	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
Voos et al., 2020, US (47)	n = 18 Smokers: daily smokers of ≥5 TC per day, FTND≥2, eCO ≥6 ppm. Mean (SD) age: 44.1 (7), 50% females.	Vaping (n=18): 20 puffs every 30 seconds for 10 minutes of a mod type VP (The SuperCig Automatic eGo 510 Battery 910 mAh, CE4 clearomizer, 4.1 V, 3 Ω resistance) with 24 mg/mL nicotine e-liquid and different flavours. 1. Vanilla. 2. Cherry. 3. Menthol 4. Espresso. 5. Classic tobacco. 6. Smoking (n=18): ad lib use of a TC.	**Maximum plasma nicotine concentration (C_{max})** 1. Median (IQR) = 9.73 (10.4) ng/mL. 2. 21.2 (30.8). 3. 15.2 (21.2). 4. 13.1 (12.5). 5. 12.5 (12.5). 6. 29.2 (15.7). **Time to maximum nicotine concentration (T_{max})** 1. Median (IQR): 10 (3) minutes. 2. 10 (5). 3. 10 (3). 4. 10 (3). 5. 10 (3). 6. 5 (3).	Some concerns

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			Area under the concentration–time curve (AUC_{0-120}) 1. Median (IQR): 174.3 (278.5) ng/mL/min. 2. 293 (318). 3. 214.4 (415). 4. 285.7 (307.8). 5. 172 (299.9). 6. 702.6 (612.3)	
Acute exposure				
Baldassarri et al., 2018, US (48)	n = 7 Vapers (n=4): daily VP use for ≥1 past month, urinary cotinine >50 ng/mL at baseline. Mean (SD) age: 26 (4), 1 female.	1. Vaping, 8mg/mL (n = 4, , Sovapers): 10 puffs every 30 seconds for 5 minutes of a tank VP (eGo type EC battery with 3.3 V, 1000 mAh, 1.5 Ω dual-coil 510-style cartomizer) with 70%/30% PG/VG tobacco flavoured and 8mg/mL nicotine e-liquid.	**Maximum plasma nicotine concentration (C_{max})** 1. Vaping, 8mg/mL: mean (SD)= 6 (4) ng /mL; stat. sig. lower compared with TC (p=0.03). NS diff. compared with vaping, 36mg/mL (p=0.07). 2. Vaping, 36mg/mL: 12 (5); stat. sig. lower compared with TC (p=0.03).	Low

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
	Smokers (n=3): smoked >10 TC per day for the past year. eCO>7 ppm and urinary cotinine >50 ng/mL at baseline. Mean (SD) age: 45 (16), 1 female.	2. Vaping, 36mg/mL (n = 4, vapers): same puffing of the same VP with 36mg/mL nicotine e-liquid. 3. Smoking (n=3, smokers): one puff every 30 seconds for 5 minutes with 10 total puffs of a TC (Camel, Turkish and Domestic blend).	3. Smoking: 27 (2). **Time to maximum nicotine concentration (T_{max})** NS diff. between groups. 1. Vaping, 8mg/mL: mean (SD)= 4.5 (1) minutes. 2. Vaping, 36mg/mL: 5 (0). 3. Smoking: 5 (0). **Area under the concentration–time curve (AUC_{60-90})** 1. Vaping, 8mg/mL: mean (SD)= 175 (146) ng*min/mL; stat. sig. lower compared with TC (p=0.03). NS diff. compared with vaping, 36mg/mL (p=0.07). 2. Vaping, 36mg/mL: 389 (137); NS diff. compared with TC (p=0.29). 3. Smoking: 516 (101).	

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
Landmesser et al., 2019, Germany (49)	n = 25 Vapers (n=20): use of ≥1.5 mL e-liquid with nicotine per day, no other use of tobacco or nicotine products. Mean age (range): 44.6 (25-53) Smokers (n=5): regular smoking of ≥10 TC per day. Mean age (range): 38.0 (21-56)	1. Vaping, 10 W (n=10): 10 vaping sessions of 10 4-second puffs, each every 30 seconds of a tank type VP (Eleaf iStick TC 40W, Aspire Nautilus mini 1.8 Ω tank system) set to 10 W power with 50%/50% PG/VG tobacco-flavoured 12mg/mL nicotine e-liquid. 2. Vaping, 18 W (n=10): 10 vaping sessions of 10 4-second puffs every 30 seconds of a tank type VP (Eleaf iStick TC 40W, Aspire Nautilus mini 1.8 Ω tank system) set to 18 W power with 50%/50% PG/VG tobacco-flavoured 12mg/mL nicotine e-liquid. 3. Smoking (n=5): smoking 10 non-filtered TC (10 mg tar, 0.32 mg nicotine, 10 mg carbon monoxide).	**Maximum plasma nicotine concentration (C_{max})** Values of labelled + unlabelled plasma nicotine provided. 1. Vaping, 10 W: mean (SD): 1.38 (0.7) + 14.39 (10.8) ng/mL. 2. Vaping, 18 W: 1.9 (1.7) + 17.68 (16.51). 3. Smoking: 0.96 (0.27) + 35.07 (16.01). **Area under the concentration–time curve (AUC_{0-5})** 1. Vaping, 10 W: mean (SD): 0.73 (0.56) + 7.05 (5.61) ng/mL*h. 2. Vaping, 18 W: 0.89 (0.88) + 8.45 (8.12). 3. Smoking: 0.44 (0.11) + 12.02 (2.95).	Low
Solingapuram Sai et al., 2019, US	n = 17 Vapers/Dual users: using	1. Vaping (n=17): one standardised puff of vapour of a tank VP (V2 EX Blanks	**Maximum plasma nicotine concentration (C_{max})** Vaping: 30.4% lower than in smokers'	High

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
(50)	VP ≥4 times per month. 8 were currently smoking, 8 were ex-smokers and 1 was never smoker. Mean (SD) age: 43 (13), 47.1% females, 70.6% Caucasian, 17.6% African American, 5.9% of other ethnicity. The study also included data from 19 smokers from the other study (128).	refillable cartomizer coupled with a programmable air syringe pump) with 80%/20% PG/VG, 12mg/mL nicotine e-liquid, which was mixed with 11C-nicotine for PET after vaping. 2. Smoking (n=19): one standardised puff of a TC with 11C-nicotine for PET after smoking.	group. **Area under the concentration–time curve (AUC_{0-5})** Vaping: 28.9% lower than in smokers' group. Terminal elimination half-life (t1/2) 1. Vaping: mean (SEM)=27 (4) seconds. 2. Smoking: mean (SEM)=23 (3) seconds.	
Yingst et al., 2019, US (51)	n = 6 Vapers: adult pod VP users. Mean (SD) age: 37.8	Vaping (n=6): 30 puffs every 20 seconds for 10 minutes of own pod VP (JUUL or Ziip pods, 4 used mango flavour, 1 strawberry lemonade, 1 menthol) with 59mg/mL nicotine e-liquid.	**Maximum plasma nicotine concentration (C_{max})** Mean = 28.6 ng/mL. Time to maximum nicotine	Moderate

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
	(15.8), 66.7% females, 83.3% white.		concentration (T_{max}) Mean = 8.7 minutes.	
Yingst et al., 2019, US (52)	n = 24 Vapers (n=14): using a VP for ≥ 30 days in their lifetime, using a VP for ≥20 of the last 28 days, and using ≥12mg/mL e-liquid. Mean (SD) age: 34.3 (10.8), 42.9% females, 92.2% white. Smokers (n=10): data from earlier study that recruited smokers.	1. Vaping, cartridge (n=4): 30 puffs every 20 seconds for 10 minutes of own cartridge VP with 12-24mg/mL nicotine e-liquid. 2. Vaping, tank/mod (n=10): same puffing of own tank/modular type VP with 12-20mg/mL nicotine e-liquid. 3. Smoking (n=10): ad lib smoking of own TC.	**Maximum plasma nicotine concentration (C_{max})** 1. Vaping, cartridge: mean (SD)=2.8 (2.1) ng/mL. 2. Vaping, tank/mod: 11.5 (9.8); stat. sig. higher compared with vaping cartridge group (p=0.023). 3. Smoking: 25.9 (16.7); stat. sig. higher compared with both vaping groups combined (p=0.0043). **Time to maximum nicotine concentration (T_{max})** 1. Vaping, cartridge: mean (SD)= 10 (2.8) minutes. 2. Vaping, tank/mod: 12.1 (2.4); NS diff. compared with vaping cartridge group (p=0.181).	Low

Study author, year, country	Sample	Interventions/groupings	Findings	Risk of bias
			3. Smoking: 6.2 (2.9); stat. sig. faster compared with both vaping groups combined (p<0.001).	

Notes: 95% CI—95% confidence intervals; CV%--geometric coefficient of variation, eCO—exhaled carbon monoxide; FTND—Fagerström Test for Nicotine Dependence; LS—least squares; NS—non-significant; stat. sig.—statistically significant; TC—tobacco cigarette; VP—vaping product.

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6 Flavours in vaping products

6.1 Introduction

Objective

This chapter begins with a brief introduction to flavours and overview of flavour categories. The objective of this chapter is then to:

- describe the use of flavoured vaping products in England
- provide an overview of the role of flavours in vaping product use
- summarise the evidence on potential harm from flavourings in vaping products from studies identified in a systematic review

Context

Flavours play an important role in shaping consumer perceptions of food and drink, as well as perceptions of combustible tobacco cigarettes and vaping products. How an individual experiences and perceives a flavour is a combination of olfactory (smell), gustatory (taste), and airway stimulation, as well as possibly touch, visual and auditory stimuli (1). A smell is usually something we experience when we inhale a substance, and we sense the taste of a flavour when we inhale, swallow and exhale through the nose or mouth. A flavouring is an additive (or chemical) used to create a flavour (a sensory experience), for example vanilla flavour in vaping products is created by using vanillin.

Flavouring additives have a long history of safe use in a wide variety of foods, drinks and medicines. There are international standards for assessing intake levels, absorption and toxicity of thousands of individual flavours and these are what are referred to as ‘generally considered safe for ingestion’. The fact that few have been tested for their effects when inhaled is commonly cited as a cause for concern and caution regarding their use in vaping products. The routes of flavour exposure between nicotine vaping products and foods are fundamentally different: initial systemic exposure to flavours from food occurs primarily via the digestive tract, whereas for vaping products it is likely to occur via the mouth and possibly the upper respiratory tract (2). Natural flavourings are expensive and not widely available therefore most commercial flavourings are 'nature-identical', that is, chemically synthesised rather than being extracted from raw materials.

Flavour enhancers can also be added to food, drinks, tobacco and vaping products; these do not impart flavour itself but amplify the flavour that the product already has through its ingredients or flavouring additives. In the case of vaping products, extra sugars and

sweeteners or cooling agents may be added as flavour enhancers. Cooling agents have been found in some vaping products (3). Synthetic coolants produce a similar 'cooling' effect to menthol but without the odour or irritant effect of menthol. The synthetic coolant WS-3 (N-ethyl-p-menthane-3-carboxamide) has been found in some vaping products. Similar to menthol, this coolant activates the cold and menthol receptor in sensory neurons, which is known to suppress irritation from nicotine. Lower menthol levels with added WS-3 flavour enhancer have been found in some vaping products available in Europe but not in vaping products in the US and Canada. Erythropel and others (3) suggested that WS-3 may have been added to vaping products to meet flavour preferences in different global markets.

In 2014, it was estimated that there were more than 7,000 different flavourings available on the vaping product market (4). Flavour libraries have recently been developed to aid the classification of flavours of tobacco cigarettes (5) and vaping products (6, 7). Yingst and others (6) classified responses from 3,719 survey participants who reported the brand and product name of their e-liquid using open-ended questions, which were then classified by researchers into 11 categories. Whereas Krüsemann and others (7) conducted a systematic review of 28 published papers and proposed an e-liquid flavour wheel of 13 categories (including the 11 categories identified by Yingst and others) (table 1) and 90 subcategories (see image of proposed e-liquid flavour wheel).

Table 1. Flavour categories (adapted from Krüseman and others)

Main flavour category	Flavour examples
Tobacco	Tobacco
Menthol/Mint	Menthol; mint; peppermint
Nuts	Almond, hazelnut, peanut
Spices	Cinnamon, clove, nutmeg
Coffee/Tea	Cappuccino, espresso, latte
Alcohol	Bourbon, mojito, rum
Other beverages	Cola, lemonade, milk
Fruit: berries; citrus; tropical; other	Blueberry, lemon, mango
Dessert	Custard, cream, butter
Candy	Gummy bears, cotton candy, bubblegum
Other sweets	Vanilla, chocolate, caramel
Other flavours	
Unflavoured	Propylene glycol/vegetable glycerin base only

Use of flavoured e-liquids in England

Nicotine containing vaping products, including flavours, flavouring additives and enhancers, sold in the UK, are regulated by the Medicines and Healthcare products

Regulatory Agency (MHRA). The MHRA are the competent authority that vaping product manufacturers are legally required to notify regarding all ingredients, in e-liquids and emissions of each product intending to be sold in the UK, via the EU Common Entry Gate system. The flavouring additives (chemicals) contained within the flavour have to be included in the EU list of flavouring substances (Regulation EU 872/2012) and cannot include such things as:

- respiratory sensitisers
- vitamins
- stimulant additives (for example, caffeine)
- chemicals, such as diacetyl (8)

See MHRA advice on ingredients in nicotine-containing liquids.

Below the level of 0.1% of the final product formulation, MHRA will allow ingredients to be considered as confidential in the notification. As such, ingredients present at a level below 0.1% of the final formulation can be described collectively in the notification by an umbrella term such as ‘strawberry flavouring’. The notifier and flavour supplier should in particular consider the safety of the flavour ingredients when used in an e-cigarette. Where details of ingredients present at levels below 0.1% are not submitted with the notification, toxicological data on each ingredient must be provided if requested by the competent authority in the event of a safety problem with the product.

In a study published in 2021, Nyakutsikwa and others at the University of Nottingham in England (9) analysed data about ingredients and emissions reported to the MHRA via the EU Common Entry Gate system from November 2016 to October 2017. A total of 40,785 e-liquid-containing products were notified to the MHRA during this period. More than 1,500 different ingredients in e-liquids and emissions were identified. Of the 1,500 ingredients, 803 were flavourings of which 38 flavours were present in more than 10% of products. The most common flavouring was ethyl butyrate (reported in 42% of e-liquids), categorised as fruit flavour by Nyakutsikwa and others, who used the dominant taste and smell of the flavouring chemical according to information in PubChem and the flavour wheel by Krüsemann and others (7) mentioned above. Vanillin was reported in 35.3% of products, which produces a vanilla (sweet) flavour; ethyl maltol in 32.9% products, which produces a sweet taste; ethyl acetate in 31.3% (a fruity taste) and maltol in 30.6% (a sweet taste). Menthol flavourings were idented in 11% and tobacco flavouring in 5% of e-liquids. A similar study was conducted in the Netherlands by Havermans and others (10) using information about e-liquids submitted to the Dutch European Common Entry Gate system. A total of 19,266 e-liquid-products were identified, 16, 300 had flavour related information. The largest flavour categories were:

- tobacco (16%)
- fruit-other (15%)
- fruit-berries (13%)
- dessert (10%)

In an analysis of 243 e-liquids purchased in England, the US, Canada and Australia in April to September 2017 to assess whether differences in regulations were associated with differences in the chemical composition of vaping products, Fix and others (11) reported flavourings (and nicotine concentration) varied by country. One hundred and sixty-six e-liquids were purchased in London, England, 54 in the US, 15 in Australia and 10 in Canada. Analyses were performed using GC (gas chromatography) mass spectrometry. Scan data were then matched against both the National Institute of Standards and Technology and Flavour and Fragrance databases to identify the total number of chemicals, as well as known flavouring or fragrance chemicals. Flavourings were reported descriptively as their concentrations were not calculated. E-liquids purchased in England contained more identifiable chemicals than those in other countries (41.6 in e-liquids bought in England compared with 10.6 bought in the US). The average number of flavouring chemicals were:

- 15.5 in e-liquids bought in Canada
- 13.9 in e-liquids bought in England
- 10.7 in e-liquids bought in Australia
- 6.7 in e-liquids bought in the US

In the e-liquids purchased in England, isopropyl methyl ketone (described as a camphor component by the authors) was the most commonly identified flavouring chemical, identified in 99.0% of e-liquids. This was followed by:

- propyl methyl ketone (which produces a sweet, fruity and banana-like flavour) in 98.4% of liquids
- acetone alcohol (described as ‘sweet, slightly green, burnt’) in 88.0%
- ethyl acetate (described as ‘ethereal, fruity, sweet with a grape and cherry nuance’) in 80.7%
- menthol (described as having cooling, mentholic, minty components) in 65.1%

As the flavouring concentrations were not measured it is unclear of what potential effect they may have. It was also unclear why the overall number of chemicals were higher in e-liquids purchased in England than those from other countries. The authors suggested it is possible that it derives from the mandate in England that nicotine-containing flavouring additives (and other chemicals) adhere to food standards, where constituents at levels above 0.1% have to be reported on the label, and those less than 0.1% are considered confidential. Manufacturers of e-liquids may use lower concentrations of a greater number of ingredients to adhere to this threshold to protect their business (in the case of 'trade secrets').

Regarding the prevalence of use of flavours in vaping products in England, in our initial evidence review of vaping (in 2015) (12), we reported that tobacco flavoured e-liquid was the most common flavour used by adults in England who currently vaped, followed by fruit then menthol. In subsequent evidence reviews (13-15), we reported that fruit followed by tobacco then menthol/mint have been the most popular flavourings. Between 2015 and 2021, fewer than 3% of vapers reported they used unflavoured e-liquids (12-15). As reported in chapter 4, fruit flavour remains the most popular among adults and for the first time, menthol/mint is slightly more popular than tobacco flavour, with preferences differing according to age and smoking status (chapter 4, figure 15, table 11).

The preference for non-tobacco flavours is consistent with the international literature. For example, a cross-sectional survey by Gravely and others (16), conducted in 2018 with 1,603 adults from Canada and the US who vaped at least weekly, found that the most common flavours were fruit, then tobacco, followed by menthol/mint, then candy/dessert, then tobacco/menthol: 2% reported they used unflavoured products (about two-thirds used a non-tobacco flavour). Flavour preferences may change between when an individual starts to vape and long-term use. Du and others (17) conducted an observational longitudinal survey of patterns of use of vaping products among 383 vapers over 3 years and 7 months. More than half of participants had changed the type of flavour they used between baseline and follow up. The proportion of participants who reported using a tobacco flavour in the first wave of the survey (26.6%) significantly reduced to 11.2% in the second wave of the survey. Whereas participants who reported using chocolate/candy or other sweet flavours significantly changed from 16.6% at wave 1 to 29.5% at wave 2 of the survey. Among young adults (aged 18 to 30 years), preference for tobacco or menthol or mint was low at both baseline and follow-up, but chocolate/candy or other sweets became the top preferred flavour in this group at follow-up. The migration to chocolate/candy or other sweet flavours was not limited to young adults, there was an increase in chocolate/candy or other sweets preference in the 31 to 45 year old group (13.4% at baseline vs. 31.7% at follow-up). There was also an increased preference of chocolate/candy or other sweet flavours in the 46 to 60 year old group. The preference of tobacco flavour decreased nearly twofold among participants aged 60 years or younger, although tobacco and menthol or mint was still the top preferred flavour among older adults (more than (>) 60 years of age). Exclusive vapers used sweet flavours more

commonly than concurrent users of vaping products and tobacco products (31% vs. 19%). At wave 2, 57.5% of participants reported they regularly used 2 or 3 flavours and 40.7% used 4 or more; only 1.8% said they only used one flavour on a regular basis.

UK population level data about e-liquid flavour preferences among young people (11 to 19 years of age) who currently vape have only recently been collected and these data were included in our 2021 evidence review (15) for the first time. As reported in chapter 3, in the past 2 years, fruit followed by menthol/mint have been the most popular flavours among young people who currently vape. Candy/dessert flavours have been the third most popular choice for young people aged 11 to 18 years and tobacco flavour the third most popular choice for 16 to 19 year olds. Unflavoured products were used by 0.7% of 11 to 18 year olds who currently vaped at least monthly and 1.7% of 16 to 19 year olds who had vaped in the last 30 days.

The preference for non-tobacco flavours among young people is consistent with the international literature for example, Park-Lee and others (18) or Schneller and others (19). The Centers for Disease Control and Prevention and the Food and Drug Administration analysed nationally representative data from the 2021 National Youth Tobacco Survey. This is a school-based, cross-sectional, self-administered survey of 20,413 middle and high school students in the US. It found 11.3% of high school students and 2.8% of middle school students reported current use of vaping products. Most (84.7%) reported using flavoured vaping products; the most commonly used flavour among all grades of students was fruit, followed by candy, desserts, or other sweets, then mint and menthol (18). In a separate nationally representative survey conducted by Schneller and others (19), using wave 3 data from the Population Assessment of Tobacco and Health (PATH) Study, use of different types of flavours and flavour combinations was reported among 415 people aged 12 to 17 years of age who had used a vaping product in the previous 30 days. Among the 226 youth participants (51.6%) who reported using only one flavour category, the top 3 flavour categories reported included fruit (52.8%), candy/desserts/other sweets (24.4%), and menthol/mint (10.8%) Just under half (45.5%) reported 2 or more flavour categories. Fruit flavour was in 9 out of the top 10 flavour combinations, while candy/desserts/other sweets appeared in 7 out of the top 10 flavour combinations. Clove/spice was the least popular individually reported flavour category (0.8%) and did not appear in any of the top 10 flavour combinations. Data from wave 2 of the PATH survey (2015 to 2016) (20) suggest flavours used among young people are fairly stable over time, but with slightly fewer young people reporting fruit alone (55% in wave 2 and 53% in wave 3) and slightly more reporting candy/other sweets alone (21% in wave 2 and 24% in wave 3).

Role of flavourings in vaping products

Flavourings in vaping products have attracted a great deal of debate, particularly about non-tobacco flavours. There is concern that fruit, dessert and other sweet flavours may make vaping appealing to people who have never smoked, especially young people,

thereby drawing them into regular nicotine use through vaping and smoking (21). Non-tobacco flavours such as menthol or sweet flavours may mask the inherent aversiveness of inhaling nicotine (22) and therefore make it easier to start vaping (23). There is concern that thermal degradation of flavour chemicals due to the heating may cause harm (24). There is also concern that some flavouring chemicals can promote the production of free radicals, including ethyl maltol, linalool, and piperonal, though some have also been found to inhibit free radical production, for example ethyl vanillin (25).

Some jurisdictions have banned non-tobacco flavours, though evidence of the effect of such bans among people who vape is lacking. Surveys that have assessed adult vapers views about their anticipated reactions to hypothetical bans suggest that the majority of study participants oppose a flavour ban (26), around a third anticipate they would find a way to obtain their preferred flavour if banned, 10 to 17% would return to smoking (17, 26) and a third would add their own flavouring agents (17). There is also some evidence that banning flavours in tobacco cigarettes and vaping products flavour may have the unintended consequence of increased uptake of smoking in young people (27).

There are several reasons why e-liquid flavours may be of help for adults transitioning from smoking to vaping. The palatability of flavourings and the range of available flavourings have been cited as motivators for initiation and persistence of vaping among adults who smoke. Use of flavours other than tobacco is associated with greater satisfaction and enjoyment of vaping (16, 17). Satisfaction, pleasure, and enjoyment with vaping are likely to be key factors in helping people transition from smoking to vaping and continuing to vape (28, 29), as well as nicotine delivery and relief of the urge to smoke (30). Non-tobacco flavours play a role in helping people switch from smoking to vaping. In a longitudinal study, using the PATH survey including 17,929 respondents aged 12 to 54 years (collected from 2013 to 2018), uptake of vaping was associated with increased smoking initiation in 12 to 24 year olds but also increased cessation among 25 to 54 year olds who smoked at baseline. Flavoured vaping products (‘menthol, mint, clove, spice, fruit, chocolate, alcoholic drinks, candy, or other sweets’) was not associated with greater youth smoking initiation than vaping tobacco flavoured products but was associated with greater adult smoking cessation; among adults (aged 25 to 54 years) who smoked at baseline and began vaping; the odds of cessation for those who used non-tobacco flavours were 2.3 times that of those who used tobacco-flavoured vaping products (31).

In another longitudinal study by Li and others (32), 886 people who were concurrent vapers and smokers, surveyed in 2016 and 2018 in England, Australia, Canada and the US, found that vapers who used sweet flavours (which included 11 different flavour groups such as fruit, candy, desserts, chocolate, clove or other flavours) were more likely to transition away from smoking compared to those who used tobacco flavoured or unflavoured products. If use of sweet vaping flavours is more desirable, as shown by other studies (16) then people are more likely to continue to use a product they like and reduce the chance of relapsing back to smoking, which may happen with a less desirable product.

Li and others (32) also suggested that the distinct differences between sweet flavours and the taste of smoking (tobacco flavour) may support maintenance of quit attempts. However, because of the study designs of the above studies, it is currently unknown if these observed associations are due to self-selection or causal.

Cancer Research UK (33) conducted a rapid review of the evidence of:

- the appeal of flavours to adult smokers
- flavours smokers use to initiate vaping and following quitting smoking
- the role of flavours in successful cessation attempts and relapse prevention

A scoping search was conducted up to January 2020 using PubMed, the Cochrane and Web of Science databases and was not peer reviewed. Its purpose was to rapidly scope the literature to inform England's Chief Medical Officer. Inclusion criteria for the review were:

- qualitative research into the appeal of flavours
- survey data on use of e-cigarette flavours
- randomised control trial data on e-cigarette flavours and smoking cessation

Twenty-two studies were included in the review. Davies and others (33) reported that survey data suggested that non-tobacco flavours are a common reason for initiation, continued use and high satisfaction with vaping. Qualitative studies suggested that flavours (not specified) improve user satisfaction with the product and may be viewed as a tool by some to prevent weight gain. The authors (33) stated the evidence on flavours used when starting vaping (that is, as part of a quit attempt) versus what people continue to use following smoking cessation was limited. The evidence suggested there may be some tendency to switch flavour use over time, however findings were mixed. There was some evidence suggesting non-tobacco and non-menthol flavours may increase the success of smoking cessation, but the data were limited and of low quality. Davies and others (33) also reported their search did not identify any published studies that independently assessed producer or retail data on the sale or popularity of different e-liquid flavour categories. They argued that as these data exist but are not in the public domain, they should be provided to researchers to allow a more comprehensive assessment of any role of e-liquid flavours for smoking cessation or relapse prevention.

Public Health England recently commissioned a systematic review of the evidence on youth use of e-liquid flavours, to inform policy decision-making regarding the Tobacco and Related Products Regulations (TRPR) post-implementation review. Notley and others (34) searched the literature from 1 January 2004 to 22 September 2020 for observational, qualitative and intervention studies related to flavoured vaping products that included participants under the age of 18. The authors reported on prevalence and patterns of use of flavours, the association of flavours and uptake of vaping, uptake of smoking, smoking cessation, report of adverse effects of flavours and perceptions and experience of flavours in vaping products.

Fifty-eight studies were included in the review; 39 were cross-sectional surveys, 11 were longitudinal cohort studies and 8 were qualitative or a mixed-methods design. Studies were primarily undertaken in the US (n=48). The remaining studies were conducted in the UK (n=8), 2 studies in Korea, one in Taiwan and one in Mexico. Notley and others (34) reported there was insufficient evidence that use of e-liquid flavours was associated with uptake of smoking in young people. No studies found a clear association between flavours and cessation in youth. No studies focused on or reported adverse effects specifically related to flavoured e-liquid use. The quality of the evidence on use of flavours in vaping products by young people was low overall. The authors concluded that 'the synthesis of the existing research does not yet provide a clear understanding of the risks and benefits to young smokers and non-smokers on the role of e-liquid flavours specifically as either a route away from or towards tobacco smoking' (34).

In another study commissioned by Public Health England (published after the end of our search date for our systematic review), Dyer and others (35) randomised 84 abstinent smokers to either a flavoured or unflavoured vaping product and assessed acute general and cue-elicited cigarette craving. Both groups were supplied with a tank style vaping device filled with 50/50 propylene glycol/vegetable glycerine (PG/VG), 10 to 18mg/mL of nicotine and half of participants received 2 of 4 flavours (blackcurrant, strawberry, vanilla, or caramel) and the other half received e-liquid with no added flavourings for one week. Authors stated that tobacco and menthol flavours were not included in the study because they are 'associated with cigarettes and are typically exempt from restrictions' (35). Participants average age was 28.8 years (SD=9.9) and 45% were female. At baseline, participants smoked 11.9 cigarettes per day, had smoked for an average of 9 years and 40% had ever used a vaping product. Eligibility criteria included no interest in quitting smoking and willingness to try an unflavoured vaping product. Average, peak and cue-elicited cigarette craving did not differ between participant groups. There was no statistically significant difference in flavoured versus the unflavoured vaping products regarding enjoyment of vaping, ease of transitioning from smoking to vaping, intentions to continue using a vaping product, and intentions and motivation to quit smoking; 30.9% of participants in the flavoured group and 23.8% in the unflavoured group were abstinent at follow up. The majority of participants continued to use the vaping product (95.2% in the flavoured group and 92.9% in the unflavoured group) at follow up and reported planning to use one in the future (97.6% in the flavoured group and 90.5% in the unflavoured group).The authors pointed out that 'unflavoured' e-liquid has a natural sweetness to it because of the vegetable glycerine and participants in this study were willing to use it and did not intend to stop smoking.

6.2 Evidence on exposure and potential harm from flavourings in vaping products

Summary of previous reports

Previous evidence reviews on vaping, commissioned by Public Health England

In our 2018 report (13), we concluded that while no clear evidence had been identified that specific flavourings in vaping products posed a health risk so far, inhaled chemicals of some flavourings (in particular cinnamaldehyde) could be a source of preventable risks. We suggested further research on the presence and effects of inhaled flavourings was warranted.

The National Academy of Science, Engineering and Medicine (NASEM)

NASEM (36), which searched the literature up to August 2017, provided an overview of common flavouring chemicals, associated flavour descriptors and their inhalation toxicity (in rodents). They described 4 studies of flavouring chemicals found in e-liquids, published between 2015 and 2016. Flavouring chemicals identified in these studies included diacetyl, acetylpropionyl and acetoin, chemicals used by food manufactures to add creamy flavours like butter, caramel, butterscotch, pina colada, and strawberry to food products. Cinnamaldehyde, the main chemical in cinnamon-flavoured e-liquids, and sometimes found in tobacco, sweet and fruit-flavoured e-liquids, was cytotoxic and genotoxic and adversely affected cell processes and survival in one study. Benzaldehyde (which produces a fruity taste) was also found in one study that tested the aerosol generated from a vaping device refilled with 145 flavoured nicotine containing e-liquids. Benzaldehyde was present in 75% of 145 e-liquids, with the highest concentrations in cherry flavours. NASEM also included 2 studies which showed that the formation of aldehydes during use of vaping comes primarily from thermal decomposition of flavouring chemicals, sweeteners and flavour enhancers.

The NASEM report also included 9 human or animal cell line studies that compared different types of flavours. Four studies that exposed cells to cinnamon flavours reported a cytotoxic response. One study examined 21 e-liquids and found only coffee-flavoured e-liquid exhibited a cytotoxic effect, and this was only at the highest extract concentration. Vaping product aerosols with menthol, coffee, and strawberry flavours significantly reduced cell viability and metabolic activity compared to air controls. In another study vaping product aerosols with coffee and strawberry flavours also significantly increased cytokine levels compared to both air controls and reference combustible tobacco cigarettes. Both fruit- and tobacco-flavoured e-liquids were cytotoxic to oropharyngeal tissue, with the fruit-flavoured liquids showing a higher toxicity and DNA fragmentation compared with tobacco-flavoured liquids. Fruit-flavoured liquids showed a higher toxicity than tobacco-flavours in another study and hazelnut or lime flavours only caused a slight

non-statistically significant reduction and menthol e-liquid, the highest reduction in the proliferation of human periodontal ligament fibroblasts.

NASEM included 4 human studies that measured abuse liability related to vaping product flavours. There were 98 participants, aged 18 to 59 across the 4 studies; most were smokers in 3 studies and exclusive vapers or dual users in one study. Sweet-flavoured e-liquids produced significantly greater subjective abuse liability ratings compared with non-sweet (mint, tobacco, and menthol) e-liquids and unflavoured e-liquids. Menthol and menthol/mint flavours had higher ratings for liking than unflavoured e-liquid. In one study, participants' own usual flavoured e-liquids (sweet flavours and one participant who used tobacco/vanilla flavours) produced greater satisfaction and other indicators of potential abuse liability than e-liquids provided by the researchers (strawberry or tobacco flavours). NASEM concluded there was moderate evidence that variability in vaping product characteristics (nicotine concentration, flavouring, device type, and brand) is an important determinant of risk and severity of e-cigarette dependence.

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT)

COT (37) reviewed toxicological data for 4 commonly used flavouring compounds in e-liquids, which were:

- menthol
- vanillin
- cinnamaldehyde
- menthone

COT systematically reviewed reports from authoritative bodies that had reviewed the toxicity and human health effects of exposure to the above 4 flavourings (including NASEM), searched 2 databases (Scopus and PubMed up to mid-2019) and checked reference lists of the authoritative reports and studies included from database searches.

Regarding menthol, COT (37) reported that menthol, L-menthol and DL-menthol are classified as skin and eye irritants and are not considered to be mutagenic (change the DNA of a cell) or carcinogenic (cause cancer) in the lung (though this finding was based on findings from tobacco smoking studies). COT suggested that menthol-related bronchodilation (opening of the airways), antitussive (cough suppressant) effects, decreased inhalation rate, and mucus production are likely to be due to menthol’s known irritant effects. They reported there are uncertainties regarding menthol on:

- its potential to increase the risk of infection or action of irritants in the open airways

- the extent of its effects on lung clearance
- the relevance of the formation of metabolites and breakdown products at high temperatures
- whether the metabolites and breakdown products are different from degradation products formed in cooked foods
- whether there are differences in metabolism following oral exposure compared to inhalation exposure

Regarding vanillin, COT (37) suggested this flavouring may act as an airway irritant in vapers. Overall, it did not consider it to be mutagenic. There were no inhalation studies of the carcinogenicity vanillin identified.

Regarding cinnamaldehyde, COT (37) reported it is a skin and eye irritant in animals and humans. It also suggested it may be an airway irritant in vapers. There was convincing evidence for skin sensitisation, which may indicate a potential for respiratory sensitisation, and the Committee believed that this was of concern. Overall, cinnamaldehyde was not considered to be mutagenic. No repeat dose, reproductive or carcinogenicity studies carried out with cinnamaldehyde via the inhalation route were identified.

Regarding menthone (a constituent in peppermint), the availability of data regarding inhalation toxicity and thermal decomposition of menthone was limited. The Committee concluded that overall, there is a large data gap regarding repeat dose inhalation toxicity for menthone.

In addition to the above, COT (37) highlighted several gaps in the data about flavourings such as the potential safety of co-exposure to flavouring compounds, either within a single e-liquid or resulting from the practice of mixing e-liquids. The Committee also reported there also remains some uncertainty about the temperature to which e-liquids may be heated when vaping, and to what extent this may be affected by customisation of vaping devices by individual users. COT's overall conclusion about flavourings was that:

> "The use of a wide range of flavouring products in e-liquids, for which data on toxicity by inhalation, particularly of any thermally-derived products, are generally not available, is an area of uncertainty. While there is currently no information that this is leading to adverse effects on human health, this is an important data gap."

COT (37) has devised a framework to aid risk assessment of flavouring compounds via inhalation exposure and recommended it should be considered by regulators at the time of product notification. The framework includes several steps designed as a set of principles to guide the risk assessment process. They recommend existing data or non-animal

approaches should be used to inform each step where possible (see COT framework for risk assessment of flavouring compounds).

6.3 Findings from the systematic review

Studies in humans

As described in chapter 2 (methods) the literature was searched from August 2017 to July 2021. Building on the findings in the nicotine chapter, we were interested to know what effect does exposure to flavourings in vaping products, with or without nicotine, have in humans, cells and animals. We identified 6 studies in humans (17, 38-42), 2 of which were funded by the tobacco or vaping industry (39, 41) (appendices: table 5). Two studies focused on the role of flavourings in the delivery of nicotine in cross-over studies (38, 39); as well as their pharmacokinetic data, given there were only 2 studies we include their findings on subjective effects for completeness. Three studies focused on biomarkers of exposure and used a randomised, open-label, parallel-cohort design (41), cross-over design (42) and a nationally representative cross-sectional survey (43). One study was a longitudinal survey of patterns of flavour use and self-reported adverse effects of flavours (17).

All studies were conducted in the US. For the studies assessing pharmacokinetics and subjective effects, sample sizes ranged from 18 (38) to 66 (39); participants were in their 40s and half were female. For the studies of biomarkers of exposure, sample sizes ranged from 36 (42) to 211 (43). Average ages were 35 to 39 years (41, 42) and not reported for Smith and others (40). Study participants were regular smokers with little experience of vaping, other than participants in the study by St. Helen and others (42) who recruited dual users and Smith and others (43) who included exclusive vapers (past smoking status not reported). Most studies were laboratory-based, and exposure to vaping was done under controlled conditions.

Risk of bias

The tools we used to assess the risk of bias of studies are reported in chapter 2 (methods) and ratings are included in tables 2 to 5 in the appendices, with ratings added to table 2 in this chapter. Risk of bias for the cross-over studies were rated as ‘some concerns’ and the cross-sectional studies as of moderate quality.

Pharmacokinetic and subjective effects in studies that compared different flavours

Goldenson and others (39) conducted a 7-arm, randomised, open-label, within-subjects crossover study. This was to assess the pharmacokinetics, product liking, satisfaction and

urge to smoke of a nicotine pod device (JUUL) in one nicotine concentration (59mg/mL) and 4 flavours (Virginia tobacco, mango, mint and creme), compared with participant's usual brand of cigarette, a comparator vaping device (VUSE Solo; 48mg/mL of nicotine, tobacco-flavour), or mint nicotine gum (4mg) (table 2). The study included 66 smokers, half of whom were female and their mean age was 41 years of age. Participants vaped under controlled use conditions (10 total puffs from the vaping products and cigarettes, 3 seconds puffs in duration, taken at approximately 30 second intervals). For sessions involving the nicotine gum, participants were instructed to use the 'chew and park' method for 30 minutes. Plasma nicotine pharmacokinetics at multiple times for up to 2 hours after each product was measured as well as subjective effects. Maximum plasma nicotine levels, rate of plasma nicotine rise, overall nicotine exposure, relative to baseline, subjective liking and satisfaction of vaping products were significantly lower for the JUUL vaping products and nicotine gum than the participants usual brand of cigarettes, and greater for the JUUL products than nicotine gum. Nicotine pharmacokinetics did not differ significantly among the Virginia tobacco, mint and mango flavours. Mint and mango were rated as more satisfying than Virginia tobacco and creme. Nicotine uptake from the creme flavour was significantly lower than the 3 other JUUL flavours. The authors suggested the observed differences in nicotine pharmacokinetics resulted from the amount of nicotine that was consumed, as the consumption of creme flavoured e-liquid was significantly lower than the 3 other JUUL flavours.

Voos and others (38) assessed the effect of vaping 5 different e-liquid flavourings on nicotine delivery, puffing topography, subjective effects, and user satisfaction. The study included 18 daily cigarette smokers who were also non-regular vapers (table 2). Half the sample were female, their average age was 44 years, and they smoked an average of 13 cigarettes per day. Participants smoked one cigarette of their own brand, ad libitum, during an initial visit. They returned 5 times (one week apart) and undertook a controlled puffing session for 10 mins, puffing every 30 seconds (total of 20 puffs) using a tank style vaping device. This was refilled with nicotine (24mg/mL) and one of 5 different flavours: cherry, tobacco, espresso, menthol, and vanilla (in a randomised order). Vaping different flavours resulted in different plasma levels of nicotine. Cherry flavour produced the highest plasma nicotine concentration, which was not significantly different to nicotine delivery from the participants own brand of cigarette. Vanilla flavour produced the lowest plasma nicotine concentration. Participants puffed less frequently on vanilla compared to tobacco flavour. There was no significant difference between flavours in relation to the speed of nicotine delivery. After controlling for nicotine delivery, menthol flavoured e-liquid was rated as more enjoyable than vanilla and tobacco flavoured e-liquid. At 3 minutes post-use, there was no significant difference in reduction of withdrawal symptoms according to the flavour used or between the flavours and the combustible cigarette. Also, at 3 minutes post-use, participants felt the fewest smoking urges when using cherry compared with other flavours. Voos and others (38) suggested flavours appeared to independently affect satisfaction and subjective effects and that differences in enjoyment from flavours is not solely a product of nicotine delivery.

Differences in biomarkers of exposure to nicotine and toxicants in studies that compared different flavoured vaping products

In a randomised, open-label, parallel-cohort study, Jay and others (41) examined changes in urinary biomarkers of exposure after 5 days of nicotine-salt pod system use (JUUL), compared with continuation of usual-cigarette smoking and cigarette abstinence. The study included 90 smokers, naive to vaping. Their average age was 39 years and 45% were female (table 2). Participants were randomised to 6 cohorts (n= 15 each) and were asked to exclusively vape (ad libitum) one of 4 flavours: Virginia tobacco, mint, mango, creme, or continue their usual-brand cigarette smoking, or abstain from cigarettes. Eight non-nicotine biomarkers of exposure, tobacco specific nitrosamines (NNN; NNAL) and volatile organic compounds (3-HPMA; MHBMA; S-PMA; HMPMA; CEMA; 1-OHP) reduced by an aggregate of 85.0% in the pooled vaping cohorts, reduced by 85.3% in the abstinence cohort and increased by 14.4% in the cigarette cohort. Mean total urine nicotine equivalents increased in the pooled vaping cohorts and cigarette smoking cohort and did not significantly differ. Regarding flavourings, there was sustained nicotine intake across the pooled vaping product cohorts. The Virginia tobacco and mint cohorts were lower than those observed in the mango and creme cohorts, consistent with the amount of product used. The mango cohort most closely matched the increase in total nicotine equivalents seen in the cigarette cohort, followed by creme and mint. Levels of biomarkers differed by flavour, but this was not tested for statistical significance.

St. Helen and others (42) assessed 10 urinary biomarkers of toxic and/or carcinogenic volatile organic compounds in a 2-arm counterbalanced, crossover study. The study included 36 dual users who smoked 5 or more cigarettes per day over the past 30 days, and used the same vaping product with 6mg/mL or more nicotine at least once daily for 15 or more days over the past 30 days (table 2). The average age of participants was 35.4 years and 22.2% were female. Participants used their usual brands of vaping products and cigarettes (table 2). Flavours used included dessert/candy (n=8), fruit (n=5), menthol (n=5) and tobacco (n=18).

During each arm, use of the assigned product and subjective measures were tracked by self-report for 4 days as outpatients, followed by 3 days on a research ward where product use was monitored, or abstention enforced. The first phase of each arm included a single-dose pharmacokinetic study on the first day of admission, followed by 2 days of ad libitum access to the assigned product. Further, 2 days of enforced abstention on the research ward were added immediately after the second arm to examine excretion of toxicant biomarkers during a period of no vaping or smoking. Concentrations of 9 out of 10 volatile organic compound metabolites were higher during smoking compared to vaping, except for the methylating agent’s metabolite (see chapter 6). Levels of benzene were at least 50% higher during vaping than when abstaining in 25/36 participants, (12/18 for tobacco flavour, 4/5 for menthol flavour, 5/5 for fruit flavour and 4/5 for dessert flavours) but this was not tested for statistical significance.

Using wave 2 of the PATH study, Smith and others (40) assessed whether the use of specific vaping product flavours among exclusive vapers was associated with urinary biomarkers of exposure to nicotine (cotinine) and toxicants (acrylonitrile, benzene, acrolein). The study included 211 exclusive vapers who reported their use of flavoured vaping products within the past 30 days. Demographics were not reported (table 2). Most exclusive vapers reported using only mint, clove, chocolate, and other reported flavours (31%), fruit and additional flavours (31%), followed by tobacco-only flavours (19%), and fruit-only flavours (19%). Users of fruit-only flavoured vaping products had significantly higher concentrations of the biomarker for acrylonitrile (CYMA) compared to users of a single other flavour. Concentrations of biomarkers of exposure to nicotine (cotinine), benzene (PMA), and acrolein (CEMA) did not significantly differ across flavours.

Self-reported perceived adverse effects of flavours in vaping products

Du and others (17) conducted an observational longitudinal survey of patterns of use of vaping products among 383 participants over 3.7 years (reported earlier). At follow up, the median numbers of flavours ever used was 10 (interquartile range 10 to 50). Tobacco flavour was the most common flavour used at baseline (26.6%) and chocolate/candy or other sweets at follow up (29.5%). Participants were asked to self-report perceived flavour-associated adverse reactions in the follow-up survey. Specifically, they were asked:

1. “Have you had a bad reaction to a particular e-cig liquid flavour, including an allergic reaction?”.

2. “If yes, please describe the reaction” (open-ended question).

3. “What flavour were you using at that time?” (open-ended question).

Overall, 6.9% of study participants recalled ever having had a “bad reaction” to a vaping product flavour, including:

- 2.9% (n= 11), who reported coughing/breathing problems/asthma
- 2.3% (n=9) mouth/throat irritation
- 0.8% (n=3) an allergic reaction

When asked “What flavour were you using at that time?” participants identified tobacco or menthol (n= 6), cinnamon (n= 5), fruit (n= 5), beverage (n= 2), other/not sure (n= 3), and high propylene glycol or vegetable glycerine (n= 5) (table 2). In addition, 23.7% and 31.8% reported having ever used any e-liquid that contained either popcorn flavour/diacetyl or cinnamon flavour/cinnamaldehyde or 2-methoxicinnamaldehyde, respectively. Participants who had ever used these flavours (total n= 154) were more likely than nonusers to report having had a bad reaction (13.0% vs. 2.7%; $p < 0.0001$).

To summarise, 2 studies assessing acute exposure to flavoured vaping products under controlled conditions, found the overall subjective effects for vaping products was different than that of tobacco cigarettes and nicotine delivery slower compared with tobacco smoking. There were mixed findings about whether or not the subjective effects of flavourings were due to their potential effect on enhancing nicotine delivery or increasing e-liquid consumption. Also, in these studies, cigarettes were more acceptable than flavoured vaping products and preferences for non-tobacco flavours was common across studies.

The 3 biomarker studies are limited in what they can tell us about the toxicity of flavouring in vaping products. In 2 studies, levels of tobacco specific nitrosamines and volatile organic compounds were significantly reduced in smokers and dual users who switched to vaping and similar to levels in abstinent smokers, levels slightly differed between flavours, but this was not tested for statistical significance. Users of fruit-only flavoured vaping products had significantly higher concentrations of the biomarker for acrylonitrile (CYMA) compared to users of a single other flavour in one study. In one longitudinal observational study, flavour preferences changed over time and 6.9% of vapers self-reported an adverse reaction that participants associated with flavour use. The studies that assessed pharmacokinetics and subjective effects and one of the studies that assessed biomarkers of exposure included participants who were smokers and were either new or non-regular vapers. However, previous reports have reported that experienced vapers can achieve greater increases in blood nicotine levels than naïve users under the same puffing regime (13, 36). So, it is plausible that subjective effects related to flavours may be different in experienced compared with novice vapers.

Table 2. Summary of studies including assessment of flavourings

Author, year, country	Study design, exposure length	Participants	Interventions / covariates / groupings	Study findings	Overall risk of bias[1]
Pharmacokinetic and subjective effects					
Goldenson et al., 2020, US (39)	Seven-arm within-subjects cross-over (A)	n=66 smokers mean cpd 16.5 (SD 4.5) 50% menthol smokers, 6.1% previous VP use Mean age 41.1 years, 50% female, 63.6% white, 27.3 % African-American, 6.1 % Hispanic, 3% 'other'	Participants were assigned to the following groups: Vaping: 10 x 3 second puffs of either Virginia tobacco flavour (n=63-65): JUUL pod with 59mg/mL nicotine salt. Mango (n=63-65): JUUL pod with 59mg/mL nicotine salt. Mint (n=63-65): JUUL pod with 59mg/mL nicotine salt. Creme (n=63-65): JUUL pod with 59mg/mL nicotine salt. Tobacco flavour (n=63-65): Vuse cartridge with 48mg/mL nicotine e-liquid. Smoking: Own brand TC (n=63-65): 10 3-second puffs of own brand TC.	Maximum plasma nicotine concentration (C_{max}) Stat. sig. lower for all vaping products and nicotine gum compared with TC (15.4 ng/mL). NS diff. between pod VP (6.6-8.6 ng/mL), cartridge VP (6.8 ng/mL) and nicotine gum (5.6 ng/mL). Time to maximum nicotine concentration (T_{max}) NS diff. between pod VP and TC (ps=0.26-0.94). NS diff. between pod VP and cartridge VP (ps>0.08). Stat. sig. faster in pod VP compared with nicotine gum (ps<0.001). Area under the concentration–time curve (AUC0-60) Stat. sig. lower for all vaping products and nicotine gum compared with TC. Stat. sig. higher in pod VP (Virginia Tobacco, Mango & Mint) compared with cartridge VP or nicotine gum. Stat. sig. higher in pod VP (Virginia Tobacco, Mango & Mint) compared with pod VP Crème flavour. Rate of plasma nicotine rise Stat. sig. lower for all vaping products and nicotine gum compared with TC (ps<0.004).	Some concerns

Author, year, country	Study design, exposure length	Participants	Interventions / covariates / groupings	Study findings	Overall risk of bias[1]
			Nicotine gum (n=63-65): 'chew and park' method use for 30 minutes. FU – 11 times up to 120 mins	NS diff. between pod VP and cartridge VP (ps>0.19). Stat. sig. higher in pod VP compared with nicotine gum (ps<0.02). Product liking and intent to use again: All VP + nicotine gum vs OB-TC rated significantly lower. Mango and mint JUUL VP vs Virginia tobacco VP rated significantly higher. All JUUL VP vs VUSE VP rated significantly higher. Satisfaction: JUUL VP vs VUSE VP – rated significantly higher. JUUL VP vs OB TC rated significantly lower. JUUL VP vs nicotine gum rated significantly higher, except for crème. Mango and Mint rated significantly higher than crème. Urge to smoke: OB-TC vs all products significantly reduced UTS Nicotine gum vs all VP significantly reduced UTS after 5 mins and similar for other FUs	
Voos et al., 2020, US (38)	Randomised within-subjects trial (A)	n=18 smokers (CPD: 13 (SD 5.8) 11 were previous menthol	Attended 7 weekly sessions, abstention for 8 hours prior to lab visit; ad lib smoking of OB-TC for 1st lab visit; controlled puffing session for visits 2-	Nicotine plasma levels Maximum plasma nicotine concentration (C_{max}) median ng/mL (IQR): Cherry flavour = 21.2 (30.8) Vanilla flavour: 9.73 (10.4) Menthol: 15.2 (21.2)	Some concerns

Author, year, country	Study design, exposure length	Participants	Interventions / covariates / groupings	Study findings	Overall risk of bias[1]
		cigarette smokers; 15 had previously used VP Mean (SD) age: 44.1 (7) years; 50% females.	7 using the assigned flavour for 10 mins, puffing every 30 sec (total of 20 puffs). Flavours: cherry; classic tobacco; espresso; menthol; vanilla flavours. Nicotine content 24mg/mL: The SuperCig Automatic eGo 510 Battery 910mAh. FU 14 assessments up to 120 mins.	Espresso: 13.1 (12.5) Classic tobacco) 12.5 (12.5) OB-TC 29.2 (15.7) Time to maximum nicotine concentration (T_{max}) median ng/mL (IQR) minutes: Cherry flavour = 10 (3) Vanilla flavour: 10 (3) Menthol: =10 (3) Espresso: =10 (3) Classic tobacco) =10 (3) OB-TC =5 (3) Area under the concentration–time curve (AUC0-60) Median (IQR) ng/mL/min Cherry flavour = 293 (318) Vanilla flavour: 174.3 (278.5) Menthol: = 214.4 (415) Espresso: = 285.7 (307.8) Classic tobacco) = 172 (299.9) OB-TC = 702.6 (612.3) Menthol was significantly more satisfying than tobacco flavour (p=.0128) and cherry (p=.0092). Menthol was also more enjoyable than both tobacco flavour (p=.0040) and vanilla (p=.0106) when controlling for nicotine intake.	

Author, year, country	Study design, exposure length	Participants	Interventions / covariates / groupings	Study findings	Overall risk of bias[1]
				Tobacco flavour was significantly harder to puff on than espresso, menthol and cherry flavours. Vanilla was significantly harder to puff on compared to cherry.	
Biomarkers					
Jay et al., 2020, US (41)	Randomized, open-label, parallel-cohort study (A)	n=90 healthy smokers cpd 16.2 (SD 3.6); Naive to vaping Mean age 39.1 (SD 11.4), 45% female, 80% white, 14% black or African American, 2% American Indian /Alaskan Native, 3% 'other', 96% non-Hispanic/Latino.	Participants randomised to either Virginia tobacco (n=15) Mint (n=15) Mango (n=15) Crème (n=15) OB-TC (15) Abstinence (n=15). 2 days of OB-TC use, then 5 days of ad libitum use of randomised condition. confined to an inpatient clinic for 9 days	Pairwise changes from baseline (day 5 - baseline) VT n=15 / Mint n=15 / Mango n=15 / Creme n=15 / TC / AB NNN: -11.9 (15.9) / -12.8 (7.0) / -12.4 (5.9) / -1.7 (64.4) / 14.3 (40.7) / -20.0 (27.2) NNAL: -314.2 (155.5) / -246.3 (139.0) / -340.2 (155.8) / -353.4 (116.7 / 0.05 (0.30) / -1.55 (0.73) (3-HMPA): -1.54 (0.58) / -1.52 (0.60) / -1.65 (0.60) / -1.95 (0.97) / 0.05 (0.30) / -1.55 (0.73) (MHBMA: -4.9 (4.5) / -5.5 (3.9) / -4.1 (2.5) / -6.3 (5.6) / 0.8 (1.5) / -4.3 (4.0) S-PMA: -6.8 (5.3) / -7.2 (4.8) / -5.6 (2.5) / -8.5 (7.9) / 1.2 (1.7) / -5.6 (4.3) COHb: -4.9 (1.9) / -4.8 (1.7) / -5.4 (1.5) / -5.6 (2.3) / 0.8 (1.4) / -4.6 (1.7)	Some concerns
St Helen et al., 2020, US (42)	Two arm cross-over (A)	n = 36 Dual users: ≥21 years old, smoking ≥5 TC	4 days of ad lib use of either OB VP or cigarette for home use, followed by overnight abstention and 2	No. of participants with 50% increase in VOC biomarkers during VP use vs abstinence	Some concerns

Author, year, country	Study design, exposure length	Participants	Interventions / covariates / groupings	Study findings	Overall risk of bias[1]
		per day over the past 30 days, use the same VP with ≥6mg/mL nicotine at least once daily for ≥15 days over the past 30 days. Mean (SD) age: 35.4 (11.7), 22.2% females, 61.1% white, 13.9% mixed, 11.1% Latino, 8.3% Black, 5,6% of Asian ethnicity.	days of *ad libitum* vaping or smoking in a research ward and 2 days of enforced abstention. Participants used their usual brands of VP and cigarettes, (provided by the study). Types of VP used cig-a-likes (*n* = 12 participants); fixed-power tanks (*n* = 15), variable-power tanks (*n* = 6), and pod e-cigarettes (*n* = 3) Flavours used: Dessert/candy n=8; Fruit n=5; Menthol n=5; Tobacco n=18.	Dessert /candy n=8 / Fruit n=5 / Menthol n=5 / Tobacco n=18 Acrolein (3-HMPA): 1 / 2 / 1 / 4 Acrylamide (AAMA): 3 / 3 / 3 / 9 Acrylonite (CNEMA): 1 / 0 / 1 / 0 1,3-Butadiene (MHBMA 1+2): 2 / 1 / 2 / 2 1,3-Butadiene (MHBMA 3): 3 / 2 / 3 / 6 Benzene (PMA): 4 / 5 / 4 / 12 Crotonaldehyde (HPMMA): 5 / 2 / 5 / 6 Ethylene oxide (HEMA): 1 / 2 / 1 / 1 Methylating agent (MMA): 3 / 4 / 3 / 4 Propylene oxide (2-HPMA): 2 / 5 / 2 / 10	
Smith et al., 2019, US (40)	Cross-sectional	n=211 Exclusive e-cigarette users reported their use of flavoured	(1) fruit-only, (2) tobacco-only, (3) single other flavour (including mint, clove, chocolate, and other reported flavours), and (4)	Users of fruit-only flavoured e-cigarettes exhibited significantly higher concentrations of CYMA compared to users of a single other flavour (geometric mean ratio = 2.71, 95% CI: 1.30–5.62, adjusted p-value 0.048). Concentrations of biomarkers of exposure to cotinine,	10/20

<table>
<tr><th>Author, year, country</th><th>Study design, exposure length</th><th>Participants</th><th>Interventions / covariates / groupings</th><th>Study findings</th><th>Overall risk of bias[1]</th></tr>
<tr><td></td><td></td><td>e-cigarettes within the past 30 days. Demographics not reported.</td><td>fruit + use of additional flavours.</td><td>PMA), CEMA) did not significantly differ across flavours.

<table>
<tr><th></th><th>Tobacco</th><th>Fruit</th><th>Other single flavour</th><th>Fruit & other flavour</th></tr>
<tr><td>Nicotine (cotinine)</td><td>1434.6</td><td>725.9</td><td>854.7</td><td>1256.2</td></tr>
<tr><td>Acrylonitrile (CYMA)</td><td>4.04</td><td>7.55</td><td>2.79</td><td>4.56</td></tr>
<tr><td>Benzene (PMA)</td><td>0.98</td><td>0.87</td><td>0.80</td><td>0.89</td></tr>
<tr><td>Acrolein (CEMA)</td><td>112.3</td><td>95.5</td><td>103.3</td><td>93.3</td></tr>
</table></td><td></td></tr>
<tr><td>Du et al., 2020, US (17)</td><td>Longitudinal survey (L)</td><td>N=383 adults in 2012–2014 and in 2017–2019. 86% were exclusive vapers, 13% were dual users. Mean age, 44 (SD 12) years, female, 33%.</td><td>Self-reported use of flavoured VP</td><td>Self-reported adverse reactions to flavours reported by 26 (6.9%) participants.

Use of tobacco flavour significant decreased over time. BL: 102 (26.6%), FU 43 (11.2%) (p<0.0001)
Use of chocolate/candy/sweet flavours significantly increased over time (BL: 63 (16.5%), FU:113 (29.5%) (p<0.0001).</td><td>Serious risk</td></tr>
</table>

Notes: A—acute exposure; AB—abstinent; BL—baseline; CO—carbon monoxide; FU—follow-up; OB—own brand TC—tobacco cigarette; VP—vaping product, nnVP—non-nicotine vaping product; UTS—urge to smoke.

[1] Risk of bias measured using different tools for different study designs: RCTs & cross-over studies—RoB2 risk of bias tool; non-randomised longitudinal studies—ROBINS-I risk of bias tool; cross-sectional studies—BIOCROSS risk of bias tool.

Studies in cells

Study characteristics

Our search identified 13 in vitro studies published since August 2017. Four studies assessed the effects of flavoured aerosolised vaping products compared to cells exposed to non-flavoured vaping products and/or tobacco smoke (44-47); 5 studies compared the exposure to vaping different flavours with untreated/air-controls (48-52) and 4 looked at exposure to vaping aerosol only (53-56). Three studies were funded by the tobacco industry (44-46), and the remainder were funded by grants from organisations such as the National Institute of Health, Food and Drug Administration (47-56). Details of study funding can be found in the appendices.

Summary of findings

Czekala and others (44) assessed vaping product aerosol with PG/VG, 24mg/mL nicotine with or without blueberry flavouring and had a comparison tobacco cigarette group. Iskandar and others (45) assessed vaping product aerosol generated from PG/VG alone or with nicotine and flavours (though flavour type is not reported) and had a tobacco cigarette smoke comparison group. Wieczorek and others (46) assessed 12 e-liquids and corresponding aerosols, with PG/VG and 12 or 24mg/mL nicotine with the following flavours: tobacco, gold leaf, menthol, mint chocolate, vanilla, caramel cafe, cherry, strawberry mint, berry cobbler, blueberry, glacier mint; and a tobacco cigarette comparison Tellez and others (47) assessed vaping product aerosols with and without 12mg/mL nicotine and 10 flavoured brands. These were Arctic Blast, Blue Pucker, Jamestown, Love Potion, Mardi Gras, Midnight Splash, Port Royale, Tobacco Row, Tortuga, Uptown and an unflavoured product (similar PG/VG and with or without nicotine) and a tobacco cigarette smoke comparison group.

Urena and others (48) assessed aerosols with PG/VG, 0 or 6mg/mL nicotine and either Lava Flow (strawberry, pineapple, and coconut), Very Cool (blueberry, blackberry, and raspberry), Hawaiian POG (orange, passion fruit, and guava), and American Patriots (tobacco) flavours. Khalil and others (49) assessed aerosols from citrus, ‘double apple’ and ‘Italian’ flavour without nicotine or ‘rich tobacco’ flavour with 1.6mg/mL nicotine and PG/VG. Behar and others (50) assessed 35 e-liquid refills and do-it-yourself products with a range of solvents and nicotine concentrations (0 to 24mg/mL). Flavours included fruit, tobacco, creamy/buttery, candy, mint, min-tobacco. Muthumalage and others (51) assessed JUUL pod flavours (Fruit Medley, Virginia Tobacco, Cool Mint, Crème Brulee, Cool Cucumber, Mango, and Classic Menthol) with 5% nicotine and other pod flavours: ‘Just Mango’ (strawberry and coconut) and Café Latte with 6% nicotine. Lamb and others (52) assessed Menthol or Virginia tobacco flavoured JUUL pods with 5% nicotine aerosols.

Clapp and others (53) assessed PG/VG nicotine-free vaping product with 3 different cinnamaldehyde-containing products ('Kola', 'Hot Cinnamon Candies' and 'Sinicide') with either nicotine alone (0.5mg/mL) or a mixture of 0.5mg/mL nicotine and 10 mM cinnamaldehyde. Jarrell and others (54) assessed aerosols from PG/VG with or without nicotine or maltol-flavoured PG/VG with nicotine (3.9 mM maltol and 100 µM nicotine for first-hand exposure, 3.9 µM maltol and 100 nM nicotine for second-hand exposure). Rowell and others (55) assessed 100 flavoured e-liquids and focused on banana pudding e-liquid with and without 12mg/mL nicotine. Abouassali and others (56) assessed aerosol from PG/VG alone, with 6mg/mL nicotine or with nicotine and flavourings (Vanilla custard, Hawaiian POG (passion fruit, orange, and guava) and Apple Jax (milky cinnamon apple cereal)).

The majority of studies exposed lung-derived immortalised cell lines, commonly used in toxicological and inhalation testing, including human epithelial pulmonary cells (A549 or Calu-3) (49, 50, 55), human bronchial epithelial cells (16-HBE and/or Beas-2b) (46, 51, 52, 54) and primary human bronchial epithelial cells (53, 55). Two studies used 3D models of the human respiratory and buccal epithelium (44, 45). The remaining studies used normal and cancerous oral cell lines (47, 48), monocytes (U937) (51), human embryonic stem cells and HL-1 mouse atrial cardiomyocytes (56). The air−liquid interface (ALI) system was used in 5 studies to simulate direct vaping product aerosol exposure in airway cell culture models (44-46, 48, 53). The number of flavourings, nicotine concentrations and length of exposure varied across the studies.

Three studies, funded by the tobacco industry, reported that the toxicity of flavoured vaping products was lower or absent in comparison to tobacco smoke. Czekala and others (44) reported that cells (EpiAirway) exposed to cigarette smoke reduced tissue viability and structure, increased inflammatory biomarkers and signs of oxidative stress, whereas exposure to an aerosol with PG/VG and nicotine, with and without blueberry favouring did not induce these changes. Iskandar and others (45) reported cells exposed to tobacco smoke caused tissue damage in buccal and small airway cultured cells whereas PG/VG and nicotine with and without added flavourings did not cause such damage. Vaping product exposure (with or without flavourings) triggered alterations in gene expression and secreted inflammatory mediators to a lower extent than tobacco smoke. In a study by Wieczorek and others (46), tobacco smoke induced a significant and substantial increase in cytotoxicity, mutagenicity and genotoxicity in human bronchial epithelium and cancer cells. Exposure to vaping product aerosols, with and without nicotine and in a range of flavours (tobacco, menthol, fruit and dessert), showed no mutagenic or genotoxic effects compared to tobacco smoke. Exposure to vaping product aerosols resulted in increased cytotoxicity for some flavours, but to a lesser extent than tobacco smoke. Exposure to vaping product aerosols generated from flavoured e-liquid containing 24mg/mL of nicotine was significantly more cytotoxic than aerosols generated from similarly flavoured products containing 12mg/mL of nicotine.

Muthumalage and others (51) exposed human bronchial epithelial cells to JUUL pod flavours (Fruit Medley, Classic Menthol, Cool Mint, Crème Brulee, Cool Cucumber, and Virginia Tobacco) with 5% nicotine. Other pod flavours, including 'Just Mango' (Strawberry Coconut flavour with unlisted nicotine concentration) and 'Café Latte' with 6% nicotine were also tested for their effects of oxidative stress, inflammatory response, epithelial barrier function, and DNA damage. JUUL pod flavours, Cool Mint, Crème Brulee, Cool Cucumber, and Fruit Medley, generated significantly higher reactive oxygen species (ROS) levels compared to the respective air control group. Flavours increased mitochondrial superoxide production with Classic Menthol inducing the greatest mitochondrial ROS production. Cool Cucumber significantly increased levels of the inflammatory cytokine marker IL-8 compared to the unexposed group. Similarly, Just Mango (Strawberry Coconut) also produced significantly high IL-8 levels compared to the untreated control group. Classic Menthol but not Cool Cucumber exposure significantly increased prostaglandin E2 levels compared to the untreated control in monocyte cells. Cool Cucumber and Classic Menthol did not show a significant increase in levels, whereas Just Mango (Strawberry Coconut) and Café Latte showed significantly elevated levels of Prostaglandin E2 compared to the unexposed control group in epithelial cells. Cool Cucumber, Classic Menthol, Just Mango and Café Latte flavoured pods also showed DNA damage.

Tellez and others (47) tested 10 flavoured vaping product aerosols, though their flavour types are not reported (only descriptors or brand names, for example 'Mardi Gras'). The cells (immortalised oral epithelial) exposed to tobacco smoke showed greater signs of toxicity than those exposed to vaping product aerosol. Three vaping product aerosols caused cytotoxicity and lipid peroxidation, while 9 induced oxidative stress levels up to 2.4-fold. The presence or absence of nicotine did not appear to be a factor in the level of cytotoxicity. PG/VG alone did not show any signs of cytotoxicity. Vaping product induced genotoxicity was increased up to 5-fold relative to baseline for some flavours.

Behar and others (50) exposed pulmonary and stem cells to 35 e-liquids and their aerosols. Fruit, tobacco, mint/menthol, cinnamon and creamy/buttery flavours with either PG, VG and nicotine concentrations of 0 to 24mg/mL were compared with each other. Aerosols from PG only, VG only and nicotine with PG were also tested. Cytotoxicity of the refill fluids did not change when stored for one year at 4°C and 2 lost their potency. Aerosols from the creamy/buttery flavoured refill fluids were more cytotoxic than any other flavour group. Glycerine-based refill fluids produced aerosols that were cytotoxic 91% of the time indicating that glycerine alone may be more harmful than propylene glycol or mixed solvent products.

Urena and others (48) exposed 2 human oral cell lines to vaping product aerosols with 4 flavours (a strawberry, pineapple and coconut flavour mix, a blueberry, blackberry and raspberry flavour mix, an orange, passionfruit and guava flavour mix and tobacco flavour) with no nicotine or 6mg/mL of nicotine. The strawberry, pineapple and coconut flavour mix

with 0% nicotine strength induced cytotoxicity and caused a significant increase in intracellular oxidative stress with both nicotine strengths, whereas the other flavours did not significantly induce cytotoxicity.

Khalil and others (49) exposed human pulmonary cells to vaping product aerosols with 4 different flavours ‘citrus’ flavour, ‘Italian’ flavour, ‘double apple’ flavour and ‘rich tobacco’ flavour with and without nicotine and observed a dose-dependent decrease in cell viability in all flavours, along with genotoxic and apoptotic induction.

As identified in the COT review (37) and the 2018 report commissioned by Public Health England (13), absolute harm of cinnamaldehyde-containing vaping products have been highlighted as a potential cause for concern. Three studies support this. Abouassali and others (56) exposed human and mice cells to fruit, vanillin and cinnamaldehyde flavourings with PG/VG and 6mg/mL nicotine. The authors reported PG/VG only exposure caused no effect. Vaping product aerosol with vanillin and cinnamaldehyde flavourings were more cytotoxic and had a greater effect on cardiac electrophysiological outcomes compared with fruit-flavoured aerosols. Clapp and others (53) exposed primary human bronchial epithelial cells to vaping aerosol containing cinnamaldehyde without nicotine and found this flavouring temporarily impaired airway cilia motility. Lamb and others (52) exposed cells to different concentrations of cinnamaldehyde that resulted in a dose-dependent reduction in mitochondrial function and glycolysis. Also, in this study, there was impaired mitochondrial respiration in lung epithelial Beas-2b cells after treatment with menthol-flavoured, but not tobacco-flavoured pod-based vaping product aerosol, along with altered electron transport chain protein levels.

Jarell and others (54) exposed lung bronchial epithelial cells to maltol-flavoured vaping product aerosols. The authors concluded that vaping induced changes in amino acid metabolism were exacerbated by the addition of maltol, while there was no additional effect on oxidative stress levels. Rowell and others (55) reported cells exposed to ‘banana pudding’ flavour, which contained vanillin, acutely increased cytoplasmic Ca2+ in Calu-3 airway cells, but PG/VG alone did not.

In summary, different flavourings in vaping products, particularly cinnamaldehyde and buttery flavours, have the potential to alter cellular responses compared with exposure to unflavoured PG/VG base liquids and other flavourings, but less so than exposure to tobacco smoke. The variability of flavoured e-liquids and the lack of appropriate unflavoured controls pose several challenges to data interpretation. It was not always possible to differentiate the effect of nicotine or solvents due to lack of appropriate controls, which was further complicated by variability of e-liquid composition, cell types, exposure doses and duration.

Studies in animals

Study characteristics

Our search identified 9 animal studies that examined the effect of exposing rodents to flavoured vaping products with or without nicotine and air-controls on respiratory, cardiovascular as well as other systems. Five studies were funded by independent grants (57-61) and 4 by the tobacco industry (62-65). All studies included mice except Rao and others (59), which used rats. All animals were 8 to 10 weeks old and were mostly exposed via their whole-body apart from one study that conducted nose-only inhalation exposure (59). The flavourings used varied across the studies and included blended mixes (60, 63-65) tobacco (59, 62), vanilla (57, 61), liquorice, cinnamon and creamy/buttery flavourings (58).

Summary of findings

Although multiple studies used flavoured vaping products for animal inhalation exposures, 9 studies addressed the question of whether flavourings contribute to the effects of vaping product exposure.

Three of the nine studies identified significant flavour-dependent changes as compared to an air-control group, while the corresponding carrier group did not (PG/VG with or without nicotine). Exposure to vanilla flavoured vaping products in C57BL6 mice was associated with increased lung function measurements (tissue damping, lung tidal and minute volumes) and elevated immunoglobulin IgG1 levels in bronchoalveolar lavage fluid (57). Another study showed a greater effect of tobacco flavoured vaping product exposure on airway hyperresponsiveness, mucous production, inflammation and oxidative stress in C57BL6 mice, as compared with unflavoured groups (62). Chapman and others (58) investigated the effect of flavoured vaping products on the severity of allergic airways disease in Balb/c mice, showing that cinnamon flavouring reduced airway inflammation and increased peripheral airway hyperresponsiveness, while a creamy/buttery flavour increased soluble lung collagen content. Chen and others (61) did not find a significant difference in mice exposed to a vanilla flavoured product compared with an air control condition. Relative to tobacco smoke there was a lower impact of flavoured vaping product aerosol on animal's cardiovascular function (59, 63), lung function (64), bone integrity (60) as well as ceramide profile and related enzymes in lung and plasma (65).

As described in chapter 5 (nicotine), a recent systematic review (66) assessed possible effects of nicotine concentration and flavour on abuse potential and appeal of vaping in adult current and former smokers and vapers. In relation to flavours (nicotine concentration and the interaction between nicotine and flavours is included in chapter 5), the review included 31 epidemiology or survey studies, 5 animal experimental studies and 16 experimental and clinical trials. We only include 4 of these studies in our review as we did not include self-report data (other than alongside pharmacokinetic data). However, several

studies are in the introductory sections on use of flavoured vaping products and their role in vaping. We also only included animal studies that were exposed to e-liquid aerosol and not exposed to flavoured water or injected with flavourings. The review authors concluded that flavours affect the abuse potential of vaping products by increasing product appeal, especially through the availability of a variety of flavours which can account for individual preferences. The review authors also acknowledged the need for more research on how flavours impact the effectiveness of vaping products for smoking cessation.

6.4 Conclusions

Flavourings in vaping products have attracted a great deal of debate, particularly about non-tobacco flavours. Concerns include that non-tobacco flavours may make vaping appealing to young people who have never smoked, mask the inherent aversiveness of inhaling nicotine and that thermal degradation of flavour chemicals may cause harm. On the other hand, flavourings may help adults who smoke to transition away from smoking by increasing the enjoyment and satisfaction of vaping. Previous reviews concluded that while research on flavours was very limited, the presence of some flavouring chemicals could potentially increase the risks of vaping.

As identified in earlier chapters, among adults and youth who vape in England, fruit flavour is the most popular e-liquid, followed by menthol/mint. There is some evidence to suggest that non-tobacco flavours, particularly sweet flavours, may play a positive role in helping people switch from smoking to vaping. A Public Health England commissioned systematic review of the evidence on youth use of e-liquid flavours concluded that existing research does not yet provide a clear understanding of the association of flavours in vaping products with uptake or cessation of tobacco smoking.

In 3 studies, levels of tobacco specific nitrosamines and volatile organic compounds were significantly reduced in smokers and dual users who switched to vaping products with different flavours. Biomarker levels slightly differed between flavours, but this was not tested for statistical significance. Users of fruit-only flavoured vaping products had significantly higher concentrations of the biomarker for acrylonitrile (CYMA) compared to users of a single other flavour in one study. In one longitudinal observational study, flavour preferences change over time, 6.9% of vapers self-reported an adverse reaction that participants associated with flavour use and a third had ever used a cinnamon/cinnamaldehyde containing vaping product.

Findings from the 13 cell and 9 animal studies suggest there is limited evidence that some flavourings in vaping products, particularly cinnamaldehyde and buttery/creamy flavours, have the potential to alter cellular responses but less than exposure to tobacco smoke. Exposure to unflavoured PG/VG base liquids appeared to have little or no effect. It was not always possible to differentiate the effect of nicotine or solvents from flavourings due to lack of appropriate controls and was further complicated by variability of e-liquid

composition, cell types, exposed doses and duration. Also, there was not a great deal of consistency about whether cells and or animals were exposed to e-liquids, aerosol extracts or aerosols.

There was only one study that looked at the stability of e-liquid flavourings over a period of one year (and found they were stable), but no studies conducted assessments to see if this changed the sensory properties over time.

Two studies assessing acute exposure to flavoured vaping products, under controlled conditions, found that nicotine delivery and 'positive subjective effects' (such as liking) for flavoured vaping products were lower than for tobacco cigarettes. The studies also found that positive subject effects were greater for vaping products and tobacco cigarettes, than for nicotine gum. There were mixed findings on whether or not the subjective effects of flavourings were due to nicotine delivery or increased level of consumption.

A recently published systematic review concluded that flavours affected the abuse potential (for example, liking a product and intending to use it again) of vaping products through increasing product appeal. But it acknowledged that the effect of flavours on smoking cessation needed further research.

6.5 Implications

Surveys in England should include detailed questions on the use of flavours (including mixing different flavours) in vaping products annually to track use over time. Longitudinal data in adults and youth in England would also be helpful in assessing the health effect of flavours in vaping products.

The findings of the systematic review support previous reports (our 2018 report, NASEM and COT), that cinnamaldehyde-containing vaping products continue to be a cause of concern and their inclusion of this flavouring chemical in e-liquids should be reviewed by regulatory bodies. Although there is less evidence in this systematic review, some in vitro studies suggest buttery/creamy flavoured e-liquids may also require further assessment.

A more standardised approach is needed to evaluate the risks associated with flavourings in e-liquids and aerosols in human and cell studies, independent of nicotine and PG/VG. The evaluation framework devised by COT to aid risk assessment of flavouring compounds via inhalation exposure could be considered by regulators at the time of product notification see COT framework for risk assessment of flavouring compounds.

COT also suggested that since flavourings may undergo thermal degradation or react with other constituents in e-liquids, research is needed to address the gap in our knowledge about the heating effects and to what extent this may be affected by customisation of vaping devices by individual users.

COT also suggested by gaps in the data about flavourings such as the potential safety of co-exposure to flavourings, either within a single e-liquid or resulting from mixing e-liquids, need addressing.

Also, further research is needed about the stability of flavourings over time and whether they degrade or not.

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7 Biomarkers of exposure to nicotine and potential toxicants

7.1 Introduction

The objective of this chapter is to summarise the evidence on biomarkers of exposure to vaping products in comparison with biomarkers of exposure to cigarette smoking or not using any tobacco or nicotine products (non-use). In line with other chapters, we begin by summarising the evidence from our previous evidence reviews of vaping products (1), the National Academies of Science, Engineering and Medicine review of vaping products (2) and then the Committee on Toxicity (COT) (3). We then provide the results of our systematic literature review, the methods for which are in Methods chapter.

7.2 Summary of previous reports on vaping products use association with exposure to toxicants

Previous evidence reviews on vaping, commissioned by Public Health England

The report published in 2018 (1) summarised the findings from 12 studies that assessed biomarkers of exposure, specifically NNAL, 1-HOP and 3-HPMA. Across 10 papers reporting on NNAL levels, people who used vaping products had a weighted average of 8.6% of the level of NNAL found in people who smoked; this decreased to 3.6% when restricting to those vapers who had been abstinent from smoking for at least 4 weeks. Four papers compared levels of 1-HOP. People who vaped had a weighted average of 42.4% of the levels of 1-HOP found in people who smoked. When one study that did not require smoking abstinence was excluded, this decreased to 38.1%. For 3-HPMA, 8 papers were included; they found a weighted average of 40.4% of 3-HPMA among people who vaped relative to people who smoked.

Importantly, 5 studies included people who vaped and people who were abstinent from smoking and vaping (some with short periods of abstinence) and compared both with people who smoked. Relative to people who smoked, the levels of biomarkers of exposure in people who vaped were almost the same as those in people who were abstinent. For NNAL, one study reported that levels among people who vaped were 36.6% of those of people who smoked, and 43.8% among people who were abstinent relative to people who smoked. A second study reported relative levels of 4.2% for people who vaped and 0.3% for people who were abstinent. For 1-HOP, relative levels were 30.7% among people who vaped and 29.7% among people who were abstinent. For 3-HPMA, levels in one study were 15.1% and 11.4% respectively; another study reported 58.7% and 64.1%

respectively. The similarity of the relative levels among vapers and abstainers indicates the importance of considering background levels when assessing levels of biomarkers of exposure.

In summary, the biomarker data were consistent with statistically significant reductions in exposure to harmful constituents when switching to vaping, with some biomarkers showing similar levels to non-smokers or smokers abstaining from smoking.

The 2018 report also made some summary statements on metals, aldehydes and cancer potencies (1). It was concluded that the levels of metals identified in the aerosol emitted from vaping products did not give rise to any significant safety concerns. However, product differences showed that metal emissions, however small, were unnecessary and vaping products that generate minimal metal emissions should become an industry standard. For aldehydes, although vaping products can release aldehydes and the levels can be high if the e liquid is overheated, the overheating generates an aversive taste, and this ensures that such emissions are avoided. At normal vaping temperatures, aldehyde content in the aerosol emitted from vaping products is only a small fraction of levels inhaled by smokers. Finally, a study of cancer potencies of vaping product emissions suggested that these were largely less than 0.5% of smoking.

The National Academies of Sciences, Engineering and Medicine report on the Public Health Consequences for E-Cigarettes

Most studies reviewed by NASEM to assess the toxicology of vaping (2) measured potential toxicants in e-liquids or aerosol. Based on these findings, NASEM concluded that there was conclusive evidence that, in addition to nicotine, most vaping products contain and emit numerous potentially toxic substances, and that, other than nicotine, the number, quantity, and characteristics of potentially toxic substances emitted from vaping products are highly variable and depend on product characteristics (including device and e-liquid characteristics) and how the device is operated. They also found substantial evidence that except for nicotine, under typical conditions of use, exposure to potentially toxic substances from vaping products is significantly lower compared with combustible tobacco cigarettes.

For metals, there was one study that measured chromium and nickel in urine and saliva samples. It showed that participants' internal doses of chromium and nickel were positively associated with concentrations of chromium and nickel in the aerosol emitted from vaping products. There was no comparison with smokers or non-users. Based on this study in conjunction with e-liquid and aerosol studies, they concluded that there was substantial evidence that the aerosol emitted from vaping products contains metals. The origin of the metals could be the metallic coil used to heat the e-liquid, other parts of the vaping device, or e-liquids. Product characteristics and use patterns may contribute to differences in the actual metals and metal concentrations measured in e-cigarette aerosol. They also

concluded that there was limited evidence that the number of metals in aerosol from vaping product could be greater than the number of metals in combustible tobacco cigarettes, except for cadmium, which is markedly lower in vaping products compared with combustible tobacco cigarettes.

NASEM also reviewed studies on smokers who switched to vaping and included assessment of nicotine biomarkers, exhaled nitric oxide, tobacco-specific nitrosamines and volatile organic compounds in biosamples. The studies included by NASEM were generally also included in the PHE report published in 2018 (1). NASEM synthesised the existing evidence as:

> "Several cross-sectional and longitudinal studies compared exposure to nicotine and toxicants in smokers who substituted e-cigarettes for their combustible tobacco cigarettes. All studies showed that smokers who substituted their tobacco cigarettes with e-cigarettes had significantly reduced levels of biomarkers of exposure to potentially toxic chemicals. Nicotine intake from e-cigarette devices among ex-smokers who were experienced e-cigarette users was comparable to that from tobacco cigarettes. Except for nicotine, exposure to potentially toxic substances from using e-cigarettes was significantly lower compared with smoking combustible tobacco cigarettes" (2).

Based on this evidence, they concluded that there was conclusive evidence that completely substituting vaping products for combustible tobacco cigarettes reduces users' exposure to numerous toxicants and carcinogens present in combustible tobacco cigarettes.

The Committee on Toxicity Statement on the potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems

The Committee on Toxicity (COT) systematically reviewed reports from authoritative bodies about toxicology from aerosols produced directly via machine puffing and biomarkers of exposure in vapers compared with smokers or non-users of either a vaping or tobacco product (including NASEM) (3). COT searched 2 databases (Scopus and PubMed) up to mid-2019 and checked reference lists of the authoritative reports and studies included from database searches.

For the machine puffing studies, COT noted that it was not clear how well machine puffing represented real-life use of vaping products. Particulate matter, propylene glycol, glycerol, nicotine, formaldehyde, acetaldehyde, and acrolein were detected in vaping product aerosols and exceeded the regulatory or guideline levels in air, however exposure conditions were often not comparable to human use.

COT performed risk assessments by estimating the total daily intake for each chemical (mg/kg bw/day), calculated from the highest reported level in one puff of aerosol multiplied by an estimate of likely number of puffs taken per day. These daily intakes were then compared with estimated daily exposures, either calculated from inhalation health-based guidance values if available, or as reported for other routes of exposure. In all instances the exposures were above the identified health-based guidance value.

COT also reported that under some conditions of use, e-liquid contents have been shown to undergo thermal breakdown leading to the presence of toxic degradation products such as formaldehyde in the aerosol. COT concluded that the short and intermittent pattern of exposure among vapers makes it difficult to make a robust assessment of any potential risk. As explained in the methods section, our systematic review did not include studies that focused on machine puffing.

COT also undertook a literature review of studies that assessed biomarkers of exposure to tobacco-related toxicants associated with the use of vaping products, in comparison with biomarker levels in tobacco products and in non-users of tobacco products. They identified 24 studies (the studies published after August 2017 are also included in our systematic review). The 24 studies included 7 randomised clinical studies, 3 non-randomised switching studies, 10 cross-sectional epidemiological studies, 2 studies that followed smokers switching to vaping, one review article, and one article of workshop proceedings. COT reported the majority of studies noted that exposures to tobacco-related toxicants were lower from use of vaping products than tobacco use, and some exposures were similar or higher than levels measured in non-users of vaping or tobacco products. Levels of nicotine and related metabolites were generally reported to be either lower than or equivalent to those from smoking in most studies.

7.3 Biomarkers of firsthand toxicant exposure

Biomarkers of exposure to nicotine and nicotine metabolites

Nicotine is the primary reason why people smoke, and this premise underlies the development of alternative nicotine delivery devices to combustible cigarettes which, if less harmful, could reduce the mortality and morbidity associated with cigarette smoking. We have shown (see chapters 3 and 4) that most adult vapers use vaping products containing nicotine, and, as reported by NASEM (2) and COT (3), biomarkers of exposure to nicotine were either lower than or equivalent to smoking. This section describes the results of our systematic review.

A note on tables

Tables are structured according to the design of the study (RCT, cross-over studies, non-randomised longitudinal studies and cross-sectional studies), by biosample type and by biomarker. For each study, biomarker levels are presented by user group condition (vapers/'dual users'/smokers/non-users, and for longitudinal studies an additional 'other' user group was added). For longitudinal studies, within-group biomarker percentage changes were calculated as described in Methods and table footnotes, with up or down arrows indicating the direction of change; the emboldened percentages/arrows indicate a significant change from baseline, as it was statistically tested and reported in the study. Significant differences in biomarker levels across groups, as reported in the studies, are indicated by superscripts. Please note that an absence of superscripts indicates either non-significance or that the study authors did not test comparisons for that user group. For cross-sectional studies, data cells are emboldened where the study authors carried out statistical tests across the user groups; biomarker level ratios comparing biomarker levels in vapers with levels in other user groups were calculated as described in Methods and table footnotes. Significant differences in biomarker levels across groups, as reported in the studies, are indicated by superscripts.

Given the volume of studies reported in this chapter across all biomarkers, we have restricted discussion to vapers versus smokers and vapers versus non-users only. Due to a substantial heterogeneity in how 'dual users' and 'other' user groups were defined in the included studies we did not compare these groups with vapers, smokers and non-users. However, data on 'dual users' and other comparison groups can still be found in the tables for completion.

Study characteristics

Our literature search identified 60 studies reporting on levels of biomarkers of exposure to nicotine and related metabolites (cotinine, total nicotine equivalents, 3-hydroxycotinine); 5 were randomised controlled trials (RCTs) (4-8), 9 were cross-over studies (9-17), 7 were non-randomised longitudinal studies (18-24) (Tables 1, 3, 5 and 7) and 39 were cross-sectional studies (Tables 2, 4, 6 and 8).

Of the 21 studies with more than one time point (RCTs, cross-over and non-randomised longitudinal studies) reporting on biomarkers of nicotine and nicotine metabolites (Tables 1, 3, 5 and 7), 12 were conducted in the US (4-7, 9, 10, 13, 15, 17, 19-21, 25), 4 in the UK (8, 18, 22, 24), 2 in Italy (12, 16), one in Belgium (14), one in Canada (11) and one in Poland (23). Three studies were funded by the tobacco industry (7, 8, 18, 25), with findings from one RCT being reported in 2 publications by Round and others (7) and Liu and others (25) (appendices: table 5).

Of the 39 cross-sectional studies (Tables 2, 4, 6 and 8) (26-62), 28 were conducted in the US (26-34, 36, 38, 40-42, 44, 46-50, 52, 54-56, 58-61), 5 in South Korea (39, 43, 45, 57, 62), one in the UK (51), Italy (35), Malaysia (53), Spain (37) and Turkey (63); one study used data collected in 3 countries (the US, the UK and Poland) (52). Participants in the study by Shahab and others (51) were also included in the study by Smith and others (52). Two studies were funded by the tobacco industry (26, 44).

Sample sizes of included longitudinal studies ranged from 18 in a cross-over study (17) to 520 in an RCT (4). Adult participants' ages ranged from 26.9 years (10, 13) to 47.0 years (5), and between 3.3% (13) and 60% (23) of participants were women. One RCT explored exposure to toxicants among African American and Latinx smokers who use vaping products (6) and a non-randomised longitudinal study explored changes in adolescents' (mean age 16.6, 24.9% females) vaping product use over 12 months (19).

Sample sizes of the cross-sectional studies ranged from 20 (63) to 15,099 (45). Adult participants' mean age ranged from 26.2 years (49) to 48.5 years (43) and between 2.1% (53) and 100% (47) of participants in general population studies were women. Nine studies explored levels of nicotine metabolites in specific samples: one study focused specifically on pregnant women (33), a comparison of pregnant and non-pregnant women (54), male smokers from a dental clinic (48), participants of 'American Indian' descent (31), workers of a recycling plant for exhausted oil (35), participants with and without respiratory symptoms (55), adolescents between 13 and 18 years old (50), adolescent boys who belonged to schools' baseball teams (32) and adolescents recruited from outpatient offices during scheduled visits with general paediatrician or subspecialist (28).

RCTs

A total of 1276 participants were recruited across the 5 RCTs (4-8, 25). All RCTs recruited smokers of at least 5 tobacco cigarettes per day and randomised them to either vaping, dual use, smoking, heated tobacco product (HTP) use or nicotine replacement therapy (NRT) use. Two tobacco industry-funded RCTs were conducted in confinement where participants adhered to study conditions and were followed-up for 5 (7, 25) or 7 (8) days after randomisation, and the other 3 RCTs explored participants' vaping characteristics in real-world conditions with longest follow-ups at 6 weeks (6), 8 weeks (5) and 24 weeks (4).

Cross-over studies

Nine cross-over studies (9-17) reported on nicotine metabolites of 444 participants. Studies recruited vapers, dual users, smokers or non-users and exposed participants to crossover conditions of vaping, smoking, NRT use, HTP use or non-use of nicotine products. One study (12) exposed smokers and non-users to acute vaping and tobacco cigarette smoking conditions. Most of the cross-over studies exposed participants to a

standardised single use of products while Cobb and others (15) and Czoli and others (11) had longer 5- and 7-day follow-up ad libitum product use conditions respectively.

Longitudinal studies

Seven non-randomised longitudinal studies that reported on nicotine metabolites (18-24) in total recruited 544 participants. Three studies recruited smokers (18, 21, 23), 3 recruited vapers (19, 22, 24) and one study recruited vapers and dual users (20). The follow-up length between studies ranged from 4 hours after acute exposure to vaping (20) to 24 months after smokers switched to ad libitum use of a cartridge vaping product (18).

Cross-sectional studies

Vaping, dual use and smoking definitions varied between cross-sectional studies, and most of them defined participants' smoking and vaping status based on their self-reported information. Definitions of vaping status and vaping frequency differed greatly between papers. Four studies reported metabolite levels among daily vapers (29, 31, 40, 64), 7 reported metabolite levels among those who vaped most days (27, 36, 47, 54, 55, 61, 62), 7 reported among those vaping at least weekly (26, 28, 32, 34, 46, 51, 58) and 8 among those vaping at least monthly (33, 43, 45, 56, 57, 61, 64, 65). Twelve studies did not define the frequency of vaping required for their participants in a vaping group (30, 35, 37, 38, 41, 42, 44, 48, 49, 53, 63, 66), however some instead required a minimum length of exclusive use, such as Shields and others where participants had to have been vaping for one year and exclusive vaping for at least 5 months (49). Where frequencies of smoking and vaping had been defined, 5 papers compared unequal frequencies between vapers and smokers, for example Andersen and others (26) who compared those who smoked at least 2 cigarettes per day to those who vape at least weekly. There was little consistency in the definition of dual use.

Some cross-sectional studies used the same data sources. Seven studies used data from the Population Assessment of Tobacco and Health (PATH) study (36, 47, 54, 55, 61, 64, 66), 4 used data from the US National Health and Nutrition Examination (NHANES) study (58-60, 67) and 4 used data from the Korean National Health and Nutrition Examination (KNHANES) study (39, 43, 45, 57).

Risk of bias in included studies

RCTs

All 5 RCTs that reported on nicotine metabolites were assessed to have some concerns in relation to overall risk of bias according to the RoB2 risk of bias tool (appendices: table 1). Key concerns regarding risk of bias of these RCTs were related with a lack of information on the randomisation process and pre-specified data analysis plans. On the other hand, all

5 RCTs had low risk of bias regarding measurement of outcome and missing outcome data.

Cross-over studies

Of the 9 cross-over studies that reported on nicotine metabolites, 7 were judged at some concerns of risk of bias (9, 10, 12-14, 16, 17) and 2 at high risk of bias (11, 15) according to the RoB2 risk of bias tool for cross-over studies (appendices: table 2). The high risk of bias assessment was based on potential deviations from intended interventions—both studies recruited dual users, had 5- and 7-days cross-over conditions and adherence to the non-use condition were confirmed only by self-report.

Longitudinal studies

Of the 7 longitudinal studies that reported on nicotine metabolites, 5 were judged at moderate risk of bias (18, 21-24) and 2 at serious risk of bias (19, 20) according to the ROBINS-I risk of bias tool (appendices: table 3). The 2 studies at serious risk of bias were judged to have issues with confounding in relation to participants' smoking.

Cross-sectional studies

Quality of all cross-sectional studies was assessed using Biocross quality appraisal tool (68) and is reported in appendices (appendices: table 4).

The main limitations were associated with study population representativeness (lack of sampling frame definition, sample size justification or information about response rate), lack of discussion on limitations arising from the cross-sectional study design and limited information about laboratory measurement procedures (blinded analyses, reporting on quality control procedures).

Table 1. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of nicotine among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	n = 158 Smokers: smoking ≥10 menthol (n=81) or non-menthol (n=77) TC per day and smoking the first cigarette of a day within 30 minutes. Mean age between groups ranged from 40.2 to 42.6, 32.3% females. Vapers (n=75): ad lib use of cartridge VP (Vuse solo) with tobacco (for non-menthol TC smokers, n=37) or menthol (for menthol TC smokers, n=38) flavours and 4.8% nicotine strength. Other (n=78): ad lib use of nicotine gum (White ice mint flavour), 4 mg. Adherence was enforced—the	Non-menthol, n=37: 12.9 (9.8) mg/24h (U) ↓**38.3%** Menthol, n=38: 13.4 (8.8) mg/24h (U) ↓**37.7%**	NA	NA	NA	Non-menthol, n=37: 7.9 (6.1) ↓**59.5%** Menthol, n=40: 7.2 (4.3) ↓**66.8%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		study was conducted in inpatient clinic without a possibility to use other products.					
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	n = 20 Smokers: smoking >5 TC per day for ≥12 past months. Mean (SD) age: 31 (9.7), 60% females, all Caucasian race. Vapers (n=9): at 2-week follow-up completely switched from smoking to cartridge VP (M201 Mild, 4.6 Volts, 280 mAh, 3.6-3.8 Ω) with 50/50 PG/VG ratio liquid with 11.0mg/mL of nicotine. Dual users (n=11): at 2-week follow-up continued smoking TC and using the cartridge VP.	n=9 623 µg/g creatinine (U) ↓30.8%	n=11 552 ↓57.9%	NA	NA	NA
Blood biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	n = 158 Smokers: smoking ≥10 menthol (n=81) or non-menthol (n=77) TC per day and smoking the first cigarette of a day within 30 minutes. Mean age between groups ranged	Non-menthol, n=37: 11.5 (10.4) ng/ml (BP) ↓**40.1%**	NA	NA	NA	Non-menthol, n=37: 6 (5.4) ↓**68.4%** Menthol,

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		from 40.2 to 42.6, 32.3% females. Vapers (n=75): ad lib use of cartridge VP (Vuse solo) with tobacco (for non-menthol TC smokers, n=37) or menthol (for menthol TC smokers, n=38) flavours and 4.8% nicotine strength. Other (n=78): ad lib use of nicotine gum (White ice mint flavour), 4 mg. Adherence was enforced—the study was conducted in inpatient clinic without a possibility to use other products.	Menthol, n=38: 13.0 (9.8) ng/ml (BP) ↓**36.0%**				n=40: 5.3 (4.2) ↓**75.8%**
Cross-over							
Arastoo et al., 2020, US (10)	Single use (A)	n = 100 Vapers (n=58): healthy VP users for >12 months who did not smoke. Mean (SD) age 27.7 (5.3), 32.8% females, 62.1% white, 24.1% Asian, 8.6% Hispanic. Smokers (n=42): healthy TC smokers for >12 months. Mean (SD) age 26.9 (5.6), 35.7% females, 52.4% white, 40.0% Asian, 7.1% Hispanic.	n=36 4.67 (0.71) ng/ml (BP)	NA	n=42 6.17 (0.86)[e]	NR	n=20 2.72 (1.06)[c]

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		Vapers (vapers, n=58): single use of cartridge (Greensmoke or eGo-One. 1Ω) or pod (JUUL) VP with tobacco, strawberry or mint flavoured and 1.2% nicotine vaping liquid for 60 3-second puffs every 30 seconds up to 30 minutes. Smokers (smokers, n=42): smoking own-brand TC. Non-users (vapers, n=58): single use of the same VP with 0% nicotine. Other (vapers, n=58): single use of a nicotine inhaler (Cyclone) with 5.0% nicotine.					
Chaumont et al., 2020, Belgium (14)	Single use (A)	n = 30 Vapers: former TC smokers who used VP for ≥1 year. Mean (SD) age: 38 (2), 100% males. Vapers, nicotine (n=30): 10 puffs of modular VP (Alien 2020 box mod, 60 W, 0.4 Ω, 3000 mAh) with 50/50 PG/VG ration liquid with 1.5mg/mL nicotine. Vapers, non-nicotine (n=30): same	Nicotine: 3.9 (1.7-8.2)[a2,e] ng/mL (BS) Non-nicotine: 0 (0-0.7)[a1]	NA	NA	NA	0[a1]

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		use of the same modular VP without nicotine. Non-users (n=30): sham vaping of the same modular VP.					
Haptonstall et al., 2020, US (9)	Single use (A)	n = 136 Vapers (n=49): VP use for >1 year without smoking for >1 year, Co verified (CO<10ppm). Mean (SD) age: 27.4 (5.5), 26.5% females, 59.2% Caucasian, 26.5% Hispanic, 10.2% Hawaiian, 2.1% African American. Smokers (n=40): Smoking for >1 year, CO verified (CO>10ppm). Mean (SD) age: 27.1 (5.5), 35% females, 62.5% Caucasian, 20% Asian, 12.5% African American, 5% Hispanic. Non-users (n=47): non-smokers or former smokers for >1 year, CO verified (CO<10ppm). Mean (SD) age: 26.3 (5.2), 53.2% females, 55.3% Caucasian, 19.1% Asian, 10.6% Hispanic, 8.5% African American. Vaping (n=49): vaping a cartridge or pod VP (eGo-one, 1 ohm, or JUUL) for up to 60 puffs every 30	n=22, vapers 88.77[e] ng/mL (BP) ↑6.7% n=41, non-users 2.64 ng/mL (BP, increase from 0)	NR	n=31 87.88 ↑7.2%	NR	n=19, vapers 86.03[a] ↑3.4% n=17, non-users 1.4 (increase from 0)

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		seconds with 1.2% nicotine strength strawberry flavour e-liquid (eGo-one) or 5% nicotine strength mint flavour salt (JUUL). Smoking (n=40, smokers): smoking own-brand TC in 7 minutes. Other (n=47, vapers): using nicotine inhaler with menthol flavour.					
Maloney et al., 2020, US (17)	Single use (A)	n = 18 Smokers: smoking >10 TC per day, has expired air CO>15ppm and have not tried JUUL or IQOS. Mean (SD) age: 36.8 (9.3), 44.4% female. Vaping (n=18): single monitored use of pod VP (JUUL) of mint or tobacco flavour for 10 puffs every 30 seconds and, after 25 minutes, ad lib use of the same VP for 90 minutes. Smoking (n=18): single monitored use of own-brand TC and, after 25 minutes, ad lib smoking for 90 minutes. Other (n=18): single monitored use of HTP (IQOS) of Amber or	Monitored use: 9.8 (4.9)[c] ng/mL (BP) ↑**345.5%** Ad lib use: 11.5 (9.3)[c] ng/mL (BP) ↑**167.4%**	NA	Monitored use: 20.4 (11.4)[a,e] ↑**871.4%** Ad lib use: 21 (10.2)[a,e] ↑**139.6%**	NA	Monitored use: 12.7 (6.2)[c] ↑**504.8%** Ad lib use: 11.3 (8)[c] ↑**109.3%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		Green flavour for 10 puffs every 30 seconds and, after 25 minutes, ad lib use of the same HTP for 90 minutes.					
Spindle et al., 2018, US (13)	Single use (A)	n = 30 Vapers: healthy, smoking <5 TC per day, using ≥1ml of e-liquid per day and using a VP with ≥6mg/ml nicotine for ≥3 months. Mean (SD) age: 26.9 (7.1), 3.3% females, 70% Caucasian, 13.3% Asian, 6.7% African American, 10% of other ethnicity, mean (SD) CPD: 0.03 (0.2). Vaping (n=30): two monitored sessions separated by 60 minutes using tank VP (eGo 3.3V battery with 1.5 ohm, dual-coil, 510 cartomizer, 7.3W) with 18 mg/ml nicotine of tobacco flavour for 10 puffs every 30 seconds. PG/VG ratios differed: a) 100% PG b) 20%/80% c) 2%/98% d) 55%/45% (NR)	a) 100% PG, 5 minutes after: 13.4 (8.99)[b,c] ng/mL (BP) b) 20%/80% PG/VG, 5 minutes after: 9.59 (7.95)[a] c) 2%/98% PG/VG, 5 minutes after: 8.58 (5.41)[a]	NA	NA	NA	NA

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

1 Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

2 Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

3 Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

a,b,c,d,e RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

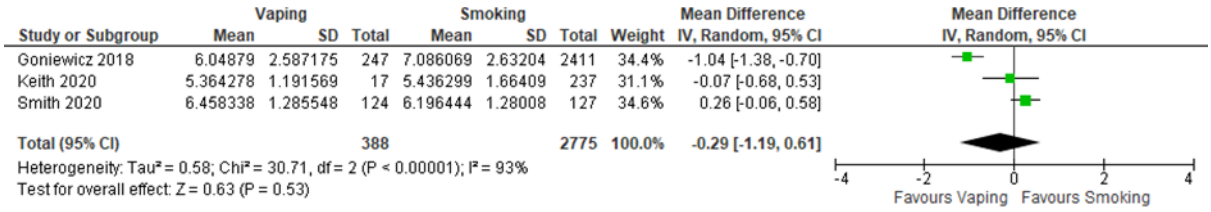
One study reported that average urinary nicotine levels were statistically significantly higher among vapers than smokers, by approximately 38% (52) (table 2). Shahab and others (51), which provided the UK data included in the Smith and others study (52), observed approximately 30% higher urinary nicotine levels among vapers than smokers, but this was not statistically significant. Frigerio and others observed higher urinary nicotine exposure among vapers than smokers (35) of around 38%, but this was not tested for statistical significance. Three other studies reported lower urinary nicotine levels among vapers than smokers (34, 36, 62) in the range between 6% and 70%, but these were not tested for statistical significance.

Rostron and others examined urinary nicotine levels among users of 2 different types of vaping products, concluding that users of open systems (tank type vaping product) were exposed to higher levels of nicotine compared with users of closed systems (cartridge or disposable vaping products) (61).

Both Rostron and Goniewicz (the largest study) studies (36, 61) used biomarker data from the Population Assessment of Tobacco and Health (PATH) survey collected between 2013 and 2016 and defined participants as vapers if they self-reported current every day or some day use of vaping product. Smith and others (52) included only more frequent users, wherein they recruited daily vapers who self-reported using at least 10 nicotine cartridges, 2 bottles of nicotine solution or 5 disposable vaping products per week for at least the past 6 months. This might have resulted in higher levels of nicotine than were reported in Goniewicz or Rostron's studies.

The pooled data across 3 cross-sectional studies (34, 36, 52) showed that the geometric mean urinary nicotine level was 25% lower among vapers than among smokers (log-transformed mean difference (LMD) = -0.29, 95% CI -1.19, 0.61; 3163 participants); the difference between groups was not statistically significant (p=0.53). There was substantial heterogeneity between studies (I^2= 93%) (figure 1). Definitions of smoking/vaping differed across the studies.

Figure 1. Meta-analysis of cross-sectional studies reporting on urinary nicotine levels between vapers and smokers



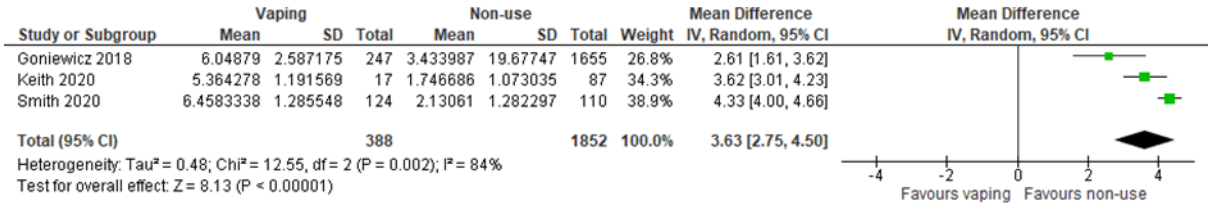
Two further small studies from the US examined blood nicotine (27, 38), both observing lower average levels of blood nicotine among vapers than smokers, although only Ghosh and others tested this comparison and did not find it to be statistically significant.

One further US study examined hair nicotine levels among vapers and smokers, reporting approximately 20% lower levels among vapers than smokers, although this was not tested for significance (56). However, the study did not report on past use of nicotine and tobacco products. Hair samples can show long term exposure to a substance, as they have much longer wash-out periods than urine, saliva or blood samples. Also, each centimetre of hair has been stated to account for approximately one month of exposure (69). Additionally, Shahab and others (51), in their UK study, also reported approximately 30% lower levels of salivary nicotine levels among vapers than smokers, although this was not statistically significant.

Of the 5 studies that compared vapers with non-users, as expected, all showed higher levels of urinary nicotine in the vapers compared to the non-users' groups, varying from over 14 to 18,000 times higher levels among vapers (34-36, 52, 62). The variation in differences is likely due to participants past nicotine and tobacco use. Frigerio and others included only those who had a urinary cotinine level below 30 µg/L as non-users, Smith and others included those who had not smoked for at least 6 months and had a carbon monoxide level below 10 ppm, Rudasingwa and others included those who were ex-smokers or had never smoked and Goniewicz and others did not define non-use.

We were able to pool data across the same 3 studies as meta-analysed for the vaper/smoker comparison of urinary nicotine levels above (34, 36, 52), finding average blood nicotine levels to be significantly higher among vapers than non-users (LMD= 3.63, 95% CI 2.75, 4.50; p=0.0001, figure 2). The geometric mean urinary nicotine levels were 37 times higher among vapers than among non-users. There was substantial heterogeneity between studies (I^2= 84%), but differences were in the same direction (figure 2). Definitions of vaping and non-use again varied across the studies.

Figure 2. Meta-analysis of cross-sectional studies reporting on urinary nicotine levels between vapers and non-users



These findings were consistent with studies comparing nicotine levels in blood and hair samples among vapers and non-users. One US study reported that average blood nicotine levels were significantly higher among vapers than non-users, by approximately 115% (38). The other US study also found higher levels but did not test for statistical significance (27). Doran and others (56) also found higher average nicotine levels in hair between vapers and non-users but did not test this for statistical significance.

Table 2. Cross-sectional studies reporting on levels of nicotine among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Urine biosample					
Frigerio et al., 2020, Italy (35)	n = 67, workers of a plant recycling exhausted oil. Age range: 27-62, 6.0% females, BMI range: 19-37 kg/m^2. Vapers (n=7): self-reported and urinary cotinine >30 µg/L. Smokers (n=21): self-reported and urinary cotinine >30 µg/L. Non-users (n=39): self-reported and urinary cotinine <30 µg/L.	Median (5th; 95th percentile): 2003 (537-4486) µg/L (U)	NA	1456 (225-5120) 1.376	0.11 (0.1-1.63) 18209.091
Goniewicz et al., 2018, US (36)	n = 5101 38% aged 35-54 years, 60% females, 61% non-Hispanic white. Vapers (n=247): every day or someday use of VP. Dual users (n=792): smoked ≥100 TC, every day or someday use of TC, VP or both. Smokers (n=2411): smoked ≥100 TC every day or someday use of TC. Non-users (n=1655): NR.	423.6 (306.7-584.9) ng/mg creatinine (U)	1318.0 (1172.8-1482.8) 0.321	1076.0 (967.7-1195.2) 0.394	31.0 (12.0-80.0) 13.665

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Keith et al., 2020, US (34)	n = 371 Mean (SD) age: 31.5 (6.8), 44.3% females, 46.1% White, 45.5% Black, 8.4% of other ethnicity. Vapers (n=17): VP use ≥7 days in last 30 days without smoking. Dual users (n=30): VP use and smoking ≥7 days in last 30 days. Smokers (n=237): smoking ≥7 days in last 30 days. Non-users (n=87): no use of TC or VP in last 30 days.	434.5 (769.5) ng/mg creatinine (U)	462.1 (639.2) 0.940	453.3 (771.7) 0.959	10.2 (15.0) 42.598
Rostron et al., 2020, US (61)	n = 751 Vapers, open system (n=205): every day or someday VP use (rechargeable and refillable). Vapers, closed system (n=72): every day or someday VP use (not rechargeable or rechargeable with cartridges). Dual users, open system (n=251): every day or someday smoking and use of open system VP Dual users, closed system (n=217): every day or someday smoking and use of closed system VP	Open system: 584.4 (383.0-891.7) µg/g (U) Closed system: 357.4 (170.0-751.2) µg/g (U)	Open system: 1019.8 (848.8-1225.3) 0.573 Closed system: 1490.3 (1169.4-1899.2) 0.240	NA	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Rudasingwa et al., 2021, South Korea (62)	n = 1586 More than 70% aged ≥30, 24.0% females. Vapers (n=24): self-reported using VP every day or sometimes. Smokers (n=403): self-reported smoking TC every day or sometimes. Non-users (n=63): self-reported non-smokers or past smokers.	Median (IQR): 339.1 (3.9; 4473.6) ng/mL (U)	NA	1121.1 (42.3; 4558.7) 0.302	3.9 (3.9; 149.5) 86.949
Shahab et al., 2017, UK (51)	n = 181 Mean (SD) age: 37.8 (11.8), 39.2% females, 72.3% White. Vapers (n=36): use VP at least weekly for ≥6 months. Dual users (n=36): smoke ≥5 CPD and use VP at least weekly for ≥6 months. Smokers (n=37): smoke ≥5 CPD for ≥6 months. Non-users (n=36): use NRT at least weekly for ≥6 months and stopped smoking ≥6 months ago.	**2.5 (1.5-4.2) nmol/mg creatinine (U)**	**4 (2.3-7.1) (U)** **0.625**	**1.9 (1.2-3.3) (U)** **1.316**	**0.8 (0.3-1.7) (U)** **3.125**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Smith et al., 2020, US, UK & Poland (52)	n = 456 Self-reported: Vapers (n=124): daily VP use for >6 past months, use of ≥10 nicotine cartridges per week or ≥2 bottles of nicotine solutions or ≥5 disposable VP per week. Dual users (n=95): use of ≥5 nicotine cartridges or ≥1 bottle of nicotine solution or ≥2 disposable VP per week and smoked ≥2 TC per day for >6 past months. Smokers (n=127): daily smoking of ≥5 TC per day for >6 past months. Non-users (n=110): no use of nicotine-containing products for >6 past months. Demographic characteristics provided by countries (US, UK, Poland) and nicotine products use status. Mean (SD) age: 40 (14). Age and gender differed statistically significantly between use groups; dual users were younger than smokers but similar in age compared to vapers and non-users. Geometric means are adjusted for age, gender, race and country of residence.	**638 (508–800)[c,d] ng/mg creatinine (U)** **1.119**	**570 (441–737)[d]** **1.623**	**393 (314–491)[a,d]** **75.772**	**8.42 (6.62–10.70)[a,b,c]**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Blood biosample					
Boas et al., 2017, US (27)	n = 27 Mean age: 29.1, 25.9% females, 70.4% White, 14.8% Asian, 11.1% Hispanic, 3.7% African American. Vapers (n=9): use VP most days for ≥1 year Smokers (n=9): use TC most days for ≥1 year Non-users (n=9): never smokers or VP users, or had quit smoking for ≥1 year	120.4 (31.6) ng/mL (BP)	NA	192. (55.8) 0.627	0 (0)
Ghosh et al., 2019, US (38)	n = 42 Mean age: 27.3, 47.6% females, 57.1% White, 40.0% Black, 9.5% Asian, 2.4% of other ethnicity. Vapers (n=14): NR. Smokers (n=13): current smoking. Non-users (n=11): never smokers.	**16.15 (17.32)**[d] **ng/ml (BP)**	NA	**29.19 (20.86)** **0.553**	**0.14 (0.19)**[a] **115.357**
Hair biosample					
Clemens et al., 2019, US (33)	n = 76, pregnant women, ≥18 years old Dual users (n=11): self-reported VP use and smoking in last 30 days. Smokers (n=27): self-reported smoking in last 30 days. Non-users (n=38): self-reported no use of TC or VP in last 30 days.	NA	**11.0 (3.8-31.3)**[d] **ng/mg (H)**	**10.6 (6.5-17.4)**[d] **1.038**	**1.1 (0.6-2.0)**[b,c] **10.0**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Doran et al., 2020, US (56)	n = 90 Mean (SD) age: 20.7 (1.7), 65% females, 47% non-Latinx white, 21% Asian, 21% Latinx, 10% other. Vapers (n=110): ≥1 days of VP use in past 30 days Dual users (n=47): ≥1 days of TC and VP use in past 30 days Smokers (n=133): ≥1 days of TC smoking in past 30 days Non-users (n=23): 0 days of use in past 30 days	0.85 (1.55) ng/mg (H)	1.20 (1.67) 0.708	1.07 (1.69) 0.794	0.17 (0.18) 5.000

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group) /(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Cotinine

Cotinine is the major metabolite of nicotine. Cotinine has average half-life of 16 hours and is considered a more stable, reliable and accurate measure of daily nicotine intake than blood nicotine, which is metabolised quicker (70).

RCTs

Two RCTs reported on urinary cotinine levels of smokers who switched to vaping product use for 6 (6) or 24 weeks (4) (table 3). Adherence to vaping product use was not enforced in both RCTs, and findings could not be meta-analysed. After 6 weeks of ad libitum use of a pod vaping product among African American and Latinx participants, median urinary cotinine levels were 10% lower compared with baseline (6). After 24 weeks of switching from smoking to ad libitum use of cartridge vaping product with 0, 8 or 36mg/mL nicotine e-liquid, urinary cotinine levels were reduced by 24.9%, 39.2% and 14.2% respectively compared with baseline, although participants within all 3 groups on average continued to smoke 7 cigarettes per day at 24-week follow-up (4); the reduction was only statistically significant within 8mg/mL vaping product users' group.

The study by Round and others was the only RCT that reported a statistically significant reduction in blood cotinine levels (by approximately 32%) after switching from smoking to vaping for 5 days in confinement (7).

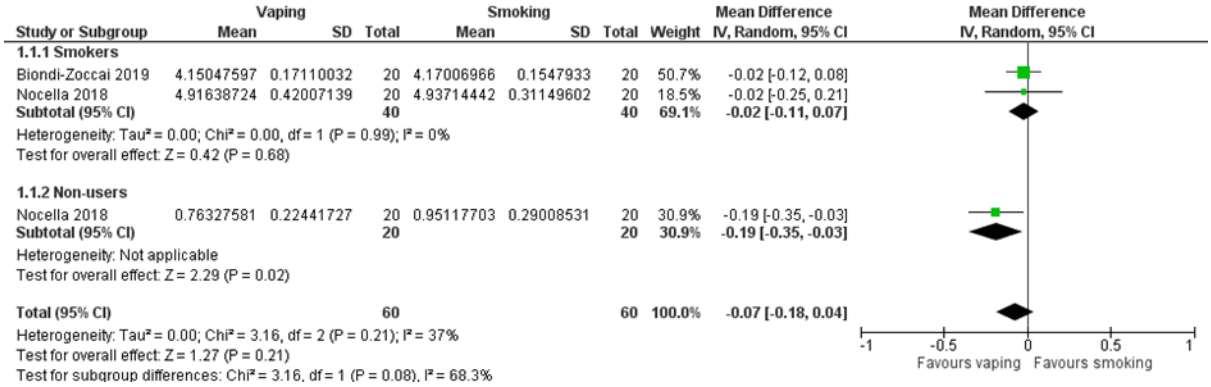
Cross-over studies

Two cross-over studies measured change in urinary cotinine levels of dual users switching to ad libitum vaping, smoking or non-use for 5 (15) or 7 (11) days (table 3). Between the 2 studies, vaping only reduced urinary cotinine levels between 29.5% and 37.5%, smoking only increased cotinine levels by 5.2% and 9.2%, and abstaining from smoking and vaping decreased urinary cotinine levels by 85.8% and 54.6%. Again, adherence was not enforced in both studies, and some participants reported smoking during vaping only or non-use.

We meta-analysed 2 cross-over studies reporting on blood cotinine levels (12, 16) following our criteria for meta-analysis (methods: table 6). Both studies were conducted by the same research team from Italy and compared acute effects of vaping 9 puffs of a cartridge vaping product with 16mg/mL nicotine e-liquid and smoking a tobacco cigarette on biomarkers of potential harm. Both studies recruited smokers and Nocella and others (12) also recruited a subsample of non-smokers. Pooled data including only smokers from 2 studies showed similar average blood cotinine levels after acute exposure to vaping compared with cigarette smoking (LMD: -0.02, 95% CI: -0.11, 0.07; 120 participants). Nocella and others tested blood cotinine levels in non-users' subsample (12), who on average demonstrated 17% lower blood cotinine levels after exposure to vaping than smoking (GMR: 0.83, 95% CI: 0.70, 0.97; figure 3). Heterogeneity was low for only-

smokers' comparison (I^2 = 0%) and slightly higher after pooling data from non-users' subsample (I^2 = 37%).

Figure 3. Meta-analysis of cross-over studies reporting on blood cotinine levels after acute exposure to vaping and smoking



Longitudinal studies

Two longitudinal studies reported on urinary cotinine levels after switching from smoking to using vaping product for 2 (23) or 4 weeks (21), and another study explored change in vaping product use among adolescents after 12 months (19) (table 3). As for urinary nicotine levels in the Goniewicz and others study (23), urinary cotinine levels also slightly differed compared with baseline among vapers (8% increase) and dual users (32.1% reduction) at 2-week follow-up, but the overall reduction in nicotine levels was not statistically significant. At 4 weeks follow-up in the Pulvers and others study (21), urinary cotinine levels also did not differ from baseline and there were no statistically significant differences at the follow-up between vapers and dual users. However, both these studies included small sample sizes (20 and 40 participants) with even smaller subsamples of exclusive vaping product users at 2- (9 out of 20 in Goniewicz and others) and 4-week (6 out of 40 in Pulvers and others) follow-ups, which might have affected their statistical power to detect differences in nicotine or other compounds' changes after switching from smoking to vaping. Vogel and others (19) followed-up adolescents (mean age 16.6) who self-reported using vaping product (at least once in the past 30 days) for 12 months to explore changes in their vaping product use. The study found that 80.3% of the initial sample continued using vaping products with an increase of daily vapers in the sample from 14.5% at baseline to 29.8% at 12-month follow-up. Median urinary cotinine level was also increased statistically significantly from baseline to 12 months as well as variability of cotinine levels within the sample, from 2.1 (IQR=35.2) ng/mL to 10.8 (IQR=79.6) ng/mL.

Two longitudinal studies from the UK (22, 24) reported on salivary cotinine level changes among vaping product users after one week and 12 months of vaping. The first study explored the effects of using different nicotine strength e-liquid and vaping product power settings on ad libitum vaping characteristics (24). The study reported compensatory puffing

behaviour among vapers using lower nicotine strength e-liquids and statistically significantly higher salivary cotinine levels among those using higher nicotine e-liquid (18mg/mL compared with 6mg/mL) and vaping products with adjustable power. The second study explored changes in salivary cotinine levels among exclusive vapers over a 12-month period (22). The study found a statistically significant decrease in self-reported nicotine strength of e-liquids, a statistically significant increase in volume of consumed e-liquid, but no significant change in salivary cotinine levels—findings that suggest vapers retained similar levels of cotinine due to compensatory puffing behaviour.

Table 3. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of cotinine among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Urine biosample							
RCT							
Cobb et al., 2021, US (4)	24 weeks (S-M)	n = 520 Smokers: smoking >9 TC per day for >1 year, had not attempted to stop smoking in the past 30 days and not planning to stop smoking in the next 6 months. Mean (SD) age: 46.2 (11.6), 59% females, 67% white non-Hispanic, 28% black non-Hispanic, 5% of other ethnicity. Vapers, 8 mg/ml (n=130): ad lib use of cartridge VP (eGo style, 3.3–4.1 volt, 1100 milliampere-h battery, 1·5 Ω) with 70%/30% PG/VG ratio and 8 mg/mL nicotine strength e-liquid of tobacco or menthol flavour selected at baseline. Vapers, 36 mg/mL (n=130): ad lib use of the same VP with	8 mg/mL, n=73 1010.55 (782.97-1304.28) ng/mg creatinine (U) ↓**39.2%** 36 mg/mL, n=79 1387.57 (1092.64-1762.11) ↓14.2% 0 mg/mL,	NA	NA	n=90 1227.72 (984.09-1531.68) ↓19.7%	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		36 mg/mL nicotine strength e-liquid. Vapers, 0 mg/mL (n=130): ad lib use of the same VP without nicotine. Non-users (n=130): ad lib use of cigarette substitute (QuitSmart) that provides a draw resistance and physical appearance of a cigarette. The substitute does not contain nicotine, tobacco and does not produce aerosol. Adherence was not enforced. At week 24, mean CPD within groups was: vapers, 8 mg/mL = 7.14, vapers, 36 mg/mL = 6.31, vapers, 0 mg/mL = 7.73, non-users = 10.44.	n=69 1115.03 (859.67-1446.23) ↓24.9%				
Pulvers et al., 2020, US (6)	6 weeks (S-M)	n = 186 Smokers: smoked ≥5 TC per day on ≥25 days of the past 30 days, smoked for ≥6 past months, had expired CO>5 ppm. Mean (SD) age: 43.3 (12.5), 40.3% females, 49.5% African American, 50.5% of Latinx ethnicity.	n=114 Median (IQR)=835 (476; 1334) ng/mL creatinine (U)	NA	n=54 1289 (643; 2078) ↑21.5%	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		Vapers (n=125): ad lib use of pod VP (JUUL) with 5% strength nicotine salts and menthol (35.2%), mango (28%), mint (19.2%) or tobacco (17.6%) flavours at baseline. Smokers (n=61): ad lib use of own brand TC. Adherence not enforced. At week 6 in vapers' group, 28.1% (n=32) were exclusive VP users, 57.9% (n=66) were dual users and 14% (n=16) were smokers.	↓10.0%				
Cross-over							
Cobb et al., 2020, US (15)	5 days (A)	n = 22 Dual users: smoking ≥10 TC per day for ≥1 year, used a cartridge VP ≥3 times per week for ≥3 months, provided an expired air CO ≥10 ppm and cotinine (U) concentration of ≥3/6 (NicAlert test strip). Mean (SD) age: 41.9 (13.2), 50% females, 50% White, 45.5% Black, 4.5% of Middle Eastern race.	n=22 EEM (SEM)=631.1 (165.2) ng/mL (U) ↓29.5%	n=22 953.0 (162.5)[d] ↑7.9%	n=22 1059.2 (162.5)[d] ↑5.2%	n=11 Mean (SEM)=106.5 (46.1)[b,c] ↓85.8%	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		Vaping: ad lib own brand VP (100% cartridge VP, 100% used 2.4%-4.8% nicotine, 81.8% menthol flavour and 18.2% non-menthol flavour). Dual users: ad lib own brand VP and own brand TC. Smoking: ad lib own brand TC. No product use: no use for the last 5-day crossover condition.					
Czoli et al., 2019, Canada (11)	7 days (A)	n=48 Dual users: smoked ≥100 TC in their lifetime, smoking ≥5 TC a day, used a VP at least once a day for the past 7 days. Mean (SD) age 35.9 (11.7); 29.2% females, 70.8% white, 29.2% other ethnicity. Vaping: ad lib own brand VP (92% tank products, 71% used ≤14 mg/mL nicotine). Smoking: ad lib own brand TC. Non-use: the last cross-over condition. Adherence not enforced. Mean CPD within cross-over conditions: vaping = 1.89,	n=48 733.7 (478.4-1125.1) ng/mg creatinine (U) ↓37.5%	NA	n=48 1282 (925.3-1776.2) ↑9.2%	n=48 533.2 (326.6-870.6) ↓**54.6%**	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		smoking = 12.35, non-use = 2.98.					
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	n = 20 Smokers: smoking >5 TC per day for ≥12 past months. Mean (SD) age: 31 (9.7), 60% females, all Caucasian race. Vapers (n=9): at 2-week follow-up completely switched from smoking to cartridge VP (M201 Mild, 4.6 Volts, 280 mAh, 3.6-3.8 Ω) with 50/50 PG/VG ratio liquid with 11.0 mg/mL of nicotine. Dual users (n=11): at 2-week follow-up continued smoking TC and using the cartridge VP.	n=9 2245 µg/g creatinine (U) ↑8.0%	n=11 1667 ↓32.1%	NA	NA	NA
Pulvers et al., 2018, US (21)	4 weeks (S-M)	n = 37 Smokers: smoking TC ≥4 days in past 30 days for ≥1 year, <25 lifetime uses of VP, not used VP for >3 days in past 30 days. Mean (SD) age: 30.1 (8.8), 27% females, 50% Caucasian, 25% Hispanic. Vapers: ad lib use of tank VP (e-Go, 3.7 volts, 650MaH) with 2.4	n=6 Median (IQR) = 266 (123.6; 386.4) ng/mg creatinine (U)	n=21 687.5 (247.3; 1193) ↑19.6%	NA	NA	n=10 361.45 (120.5; 710.5) ↓37.1%

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		Ω atomiser and preferred flavour of 12 mg/mL (2.5% at baseline) or 24 mg/mL (97.5%) nicotine strength at the last follow-up. Dual users: ad lib use of tank VP and smoking own brand TC at the last follow-up. Other: ad lib use of tank VP for first two weeks and dual use for last two weeks.	↓53.7%				
Vogel et al., 2019, US (19)	12 months (S-M)	n = 173 Vapers: self-reported use of a VP at least once in the past month and ≥10 times in their lifetime. Mean (SD) age: 16.6 (1.2), 24.9% females, 13.3% white, 10.4% of multiple ethnicities, 1.2% Asian, 1.2% African American. Vaping (n=173): self-reported use of own brand VP with some also using TC.	n=127 Median (IQR)=10.8 (79.6) ng/mL (U) ↑414.3%	NA	NA	NA	NA
Blood biosample							
RCT							
Round et al., 2019, US	5 days (A)	n = 158 Smokers: smoking ≥10 menthol	Non-menthol,	NA	NA	NA	Non-menthol,

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
(7)		(n=81) or non-menthol (n=77) TC per day and smoking the first cigarette of a day within 30 minutes. Mean age between groups ranged from 40.2 to 42.6, 32.3% females. Vapers (n=75): ad lib use of cartridge VP (Vuse solo) with tobacco (for non-menthol TC smokers, n=37) or menthol (for menthol TC smokers, n=38) flavours and 4.8% nicotine strength. Other (n=78): ad lib use of nicotine gum (White ice mint flavour), 4 mg. Adherence was enforced—the study was conducted in inpatient clinic without a possibility to use other products.	n=38: 183 (153) ng/mL (BP) ↓**32.0%** Menthol, n=38: 211 (148) ng/mL (BP) ↓**32.2%**				n=37: 117 (95) ↓**55.7%** Menthol, n=40: 110 (77) ↓**65.3%**
Cross-over							
Biondi-Zoccai et al., 2019, Italy (16)	Single use (A)	n=20 Smokers: healthy TC smokers with mean (SD) smoking time in years: 15 (12).	n=20 64.6 (11.1) ng/mL	NA	n=20 97.6 (3.4)	NA	n=20 94.5 (4.1)

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		Vapers (n=20): 9 puffs of cartridge VP (Blu pro) with tobacco flavoured 16 mg/mL nicotine strength e-liquid. Smokers (n=20): smoking a TC (Marlboro Gold). Others (n=20): using a single Amber label heets with HTP (IQOS).	(BS) ↑**104.4%**		↑**9.2%**		↑**5.2%**
Nocella et al., 2018, Italy (12)	Single use (A)	n = 40 Healthy smokers (n=20) and non-smokers (n=20). Mean (SD) age: 28 (5.3), 52.5% females, mean (SD) BMI: 23.2 (2.9). Vapers (n=40): both smokers and non-smokers used 9 puffs of a cartridge VP with 16 mg/mL nicotine and tobacco flavour. Smokers (n=40): both smokers and non-smokers smoked one TC (0.6 mg nicotine).	Smokers, n=20 149.1 (65.5) ng/mL (BS) ↑11.4% Non-smokers, n=20 2.2 (0.5) ↑15.8%	Smokers, n=20 146.3 (46.7) ↑9.0% Non-smokers, n=20 2.7 (0.8) ↑28.6%	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Saliva biosample							
Longitudinal							
Dawkins et al., 2018, UK (24)	1 weeks (A)	n = 20 Vapers: exclusive daily VP use with ≥12 mg/mL nicotine strength vaping liquid for ≥3 months. Mean (SD) age: 37.9 (10.7), 40% females, 95% white, 5% of mixed-race ethnicity. Vaping (n=20): ad lib use of a tank VP (eVic Supreme with a Nautilus Aspire' tank, 1.6 Ω) with: 1) 6 mg/mL nicotine strength and 4 volts (10 W) 2) 18 mg/mL nicotine strength and 4 volts (10 W) 3) 6 mg/mL nicotine strength and adjustable voltage (3-6V) 4) 18 mg/mL nicotine strength and adjustable voltage (3-6V)	1) 6mg/mL, fixed power, n=20 250.5 (188.2)[a2] ng/mL (S) 2) 18mg/mL, fixed power, n=20 402.5 (190)[a1] 3) 6mg/mL, adjustable power, n=20 275.0 (172.8)[a4] 4)	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			18mg/mL, adjustable power, n=20 405.2 (192.8)[a3]				
Soar et al., 2018, UK (22)	12 months (S-M)	n = 32 Vapers: VP users who did not smoke or use other nicotine. 27 out of 32 completed 12-month follow-up. Among them, mean (SD) age: 43.81 (9.2), 30% females, 85% British, 74% in paid employment. Vapers (n=27): VP users of average 13.8 mg/ml nicotine e-liquid at baseline.	n=27 415.78 (242.5) (S) ↑12.1%	NA	NA	NA	NA

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

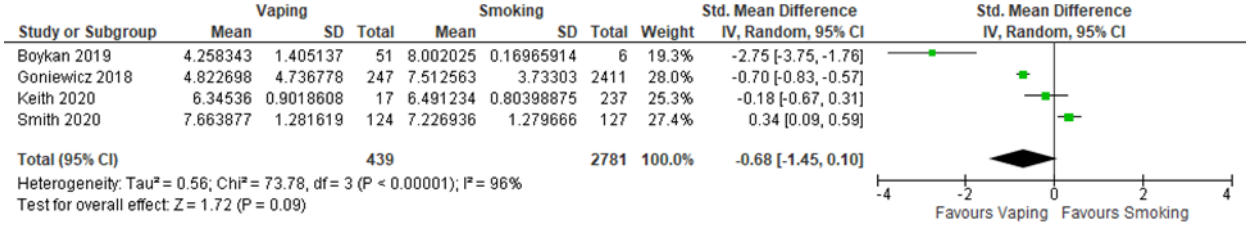
Nineteen studies reported on urinary cotinine levels across the different user groups, 7 reported blood cotinine levels, 6 reported on salivary cotinine, and 2 reported on cotinine in hair samples (table 4).

Twelve studies compared urinary cotinine exposure in vapers with smokers. Four studies found statistically significant lower average urinary cotinine levels (between 32% and 94%) among vapers than smokers (36, 47, 57, 62), and Gonzalez-Roz and others (71) also identified lower levels of around 11%, which was not statistically significant. A further 4 studies, that did not carry out statistical testing, reported lower urinary cotinine levels among vapers than smokers, ranging from 6% to 43% lower (28, 34, 35, 45). One study found statistically significantly higher (by around 55%) average levels of urinary cotinine among vapers than smokers (52), Shahab and others UK study found 27% higher levels among vapers than smokers, which was not statistically significant (51), and one further study identified 1% higher urinary cotinine levels among vapers than smokers, but this was not tested for statistical significance (29).

Based on the largest study, vapers had approximately 86% lower levels of urinary cotinine compared to smokers (45). However, this study defined vapers as those who had vaped at least once in the past month, and current smokers as those who had smoked more than 100 cigarettes in their lifetime. Most studies defined vaping as daily or some days. In the study which only included more frequent daily vapers, vapers were reported to have 55% higher (52) levels of urinary cotinine when compared to daily smokers.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), we pooled data from 4 studies (28, 34, 36, 52) finding average urinary cotinine levels to be lower among vapers than smokers. The geometric mean urinary cotinine level was 49% lower among vapers than among smokers (LMD= -0.68, 95% CI -1.45, 0.10; p=0.09; figure 4), this difference however was not statistically significant. There was substantial heterogeneity between studies (I^2= 96%).

Figure 4. Meta-analysis of cross-sectional studies reporting on urinary cotinine levels between vapers and smokers



Among the 5 studies that reported blood cotinine levels among vapers and smokers, there were very poor and or inconsistent definitions of smoking and vaping. Two studies pooled

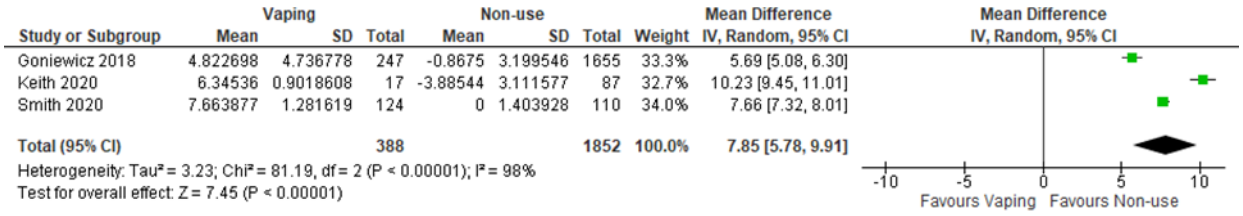
data from dual and exclusive vapers or allowed some smoking in the vaper group (40, 41), one study did not properly define vaping or smoking (38) and 2 studies used inconsistent frequencies of use across groups—for example, daily smokers compared to weekly vapers (26, 60). Overall, 3 studies that tested for statistical significance found lower average levels of blood cotinine among vapers than smokers, but all were not statistically significant (ranging from 15% to 44% lower) (38, 40, 60). A further study also reported approximately 15% lower levels of blood nicotine among vapers compared with smokers, but did not test for statistical significance (26). By contrast, Reidel and others (41) observed approximately 5% higher average blood nicotine levels among vapers than smokers, which was not statistically tested.

Similar methodological issues were found among studies reporting salivary cotinine. Four studies assessed salivary cotinine levels between vapers and smokers. One study found lower average levels among vapers and smokers (by 15%), but this was not statistically significant (48), and another study of adolescent boys, which did not test for statistical significance, observed 33% lower levels among vapers than smokers (32). By contrast, the only UK study by Shahab and others (51) reported 3% increase, and Ye and others (30) reported on average 26% higher levels of salivary cotinine among vapers than smokers, but neither of these differences were statistically significant.

For comparisons between vapers and non-users, there were 10 studies comparing urinary cotinine levels, 4 studies comparing blood cotinine levels, and 5 studies comparison salivary cotinine levels. In the 10 studies that compared urinary cotinine levels between vapers and non-users, as expected, all observed higher average levels among vapers. Four studies found statistically significantly higher average levels among vapers than non-users (36, 47, 52, 62) ranging from 230- to 2000-fold higher, and the remainder of studies did not test for statistical significance (28, 29, 34, 35, 45). Dai and others found that vapers with self-reported respiratory symptoms had 85% higher levels of urinary cotinine than non-users with symptoms, but the study did not test for statistical significance (55). In comparison, vapers without self-reported respiratory symptoms had 26% higher levels of urinary cotinine than non-users without symptoms, but again did not test for statistical significance.

Pooled across 3 studies (34, 36, 52), urinary cotinine levels were significantly higher among vapers than among non-users (LMD= 7.85, 95% CI 5.78, 9.91; p=0.0001); the geometric mean urinary cotinine level was 2565 times higher among vapers than among non-users. There was substantial heterogeneity between studies (I^2= 98%), but the direction of the difference was consistent across them (figure 5).

Figure 5. Meta-analysis of cross-sectional studies reporting on urinary cotinine levels between vapers and non-users



In the 4 studies comparing blood cotinine levels among vapers and non-users, as expected, all reported higher average levels among vapers. Three studies that statistically tested differences between the groups reported 42 to 3350 times higher blood cotinine levels among vapers than non-users by (38, 41, 42).

In the 5 studies examining salivary cotinine levels among vapers and non-uses, 4 observed 2 to 320 times higher levels among vapers than non-users—one was statistically significantly higher (48), 2 were not statistically significant (30, 51) and the fourth study among adolescent boys did not statistically test the differences (32). Rubenstein and others (50) observed very similar salivary cotinine levels among past-month adolescent vapers and non-users, which were not statistically significant.

Table 4. Cross-sectional studies reporting on levels of cotinine among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Urine biosample					
Aslan et al., 2019, Turkey (63)	n = 20 Median age: 38.5, 15% females. Vapers (n=11): NR. Dual users (n=9): NR.	685.7 (143.4) ng/mL (U)	643.1 (147.1) 1.066	NA	NA
Boykan et al., 2019, US (28)	n = 517 22% of 12-14 years old, 49% of 15-17 years old, 29% of 18-21 years old, 64% females, 66% White non-Hispanic, 9% White Hispanic, 3% African American, 11% other Hispanic. Vapers (n=51): self-reported past week use of VP. Dual users (n=9): self-reported past week use of VP and TC. Smokers (n=6): self-reported past week use of TC.	189.72 (472.49) ng/mL (U)	524.77 (708.45) 0.362	330.3 (517.84) 0.574	NA
Bustamante et al., 2018, US (29)	n = 59 Mean age: 37.9, 65.4% females, 78% White, 22% of other ethnicity. Vapers (n=19): daily VP use, ≥3 months exclusive VP use, no other tobacco use in past 6 months Smokers (n=19): smoking ≥10 CPD, no NRT, other tobacco or VP use in past 6 months. Non-users (n=18): smoked <100 TC, no tobacco or VP use in past 6 months.	17.5 (17.4) nmol/mL (U)	NA	17.3 (10.6) 1.012	0.32 (0.47) 54.688

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Dai et al., 2020, US (55)	n = 4614 % within age groups: 18-24: 34.9%, 25-34: 20.7%, 35-54: 28.7%, ≥55: 15.6%, 52.8% females, 57.8% non-Hispanic white, 18.7% Hispanic, 13.5% non-Hispanic Black, 9.9% of non-Hispanic other ethnicity. Vapers (n=153 without and n=69 with respiratory symptoms): current VP use some or every day. Dual users (n=756 w/o and n=781 with respiratory symptoms): current VP use some or every day and current some or every day use of tobacco products. Non-users (n=2008 without and n=829 with respiratory symptoms): non-users.	Without symptoms: 148.1 (72.9-301) ng/mg creatinine (U) With symptoms: 341.3 (135.3-861) ng/mg creatinine (U)	817.6 (626.1-1067.5) 0.181 2031.4 (1769-2332.6) 0.168	NA	0.8 (0.6-0.9) 185.125 2.7 (2-3.7) 126.407
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	Median (5th; 95th percentile): 1530 (1179-2772) µg/L (U)	NA	1772 (601-4000) 0.863	0.35 (0.1-1.93) 4371.429
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**124.3 (68.9-224.4) ng/mg (U)**[b,c,d]	**2858.9 (2601.9-3141.2)**[a,c] **0.043**	**1830.9 (1577.4-2125.1)**[a,b] **0.068**	**0.42 (0.36-0.49)**[a] **295.952**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	**Dual users[b]**	**Smokers[c]**	**Non-users[d]**
González-Roz et al., 2017, Spain (37)	n = 81 23.5% females. Vapers (n=39): NR. Smokers (n=42): NR.	**1891.26 (1452.11) ng/mL (U)**	NA	**2383.51 (1129.07)** **0.793**	
Hwang et al., 2021, South Korea (57)	n = 3917 Mean age within groups: vapers—37.6, dual users—35.3, smokers—43.9; % females within groups: vapers—16.1%, dual users—10.1%, smokers—12.3%. Vapers (n=52): VP use in past 30 days. Dual users (n=308): daily or sometime smokers, VP use in past 30 days. Smokers (n=3557): current smokers.	**867.7[b,c] ng/mL (U)**	**1356.4[a,c]** **0.640**	**1270.3 (17.9)[a,b]** **0.683**	NA
Keith et al., 2020, US (34)	See: Keith et al., 2020	855.8 (958.9) ng/mg creatinine (U)	851.6 (770.9) 1.005	910.9 (868.3) 0.940	2.6 (2.4) 329.154

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Kim et al., 2020, South Korea (39)	n = 7505 Mean (SE) within groups: dual users—36.7 (0.7), smokers—43.6 (0.3), non-users—39.8 (0.4). Dual users (n=337): smoked >100 TC in lifetime, currently smoke and have used a VP in the past month. Smokers (n=4079): smoked >100 TC in lifetime, currently smoke and have not used a VP in the past month. Non-users (n=3027): smoked <100 TC in lifetime or never smoked and have not used a VP for the past month.	NR	Median (IQR): 1303.4 (850.2; 1925)[c,d] µg/mL (U)	1236.1 (677.7; 1800)[b] 1.054	0.7 (0.4;1.4)[b] 1862.0
Kim et al., 2020, South Korea (43)	n = 2442 Mean (SD) age: 48.5 (15.1), 16.0% females. Dual users (n=264): smoked ≥100 TC, smoke sometimes or every day, use VP in past 30 days. Smokers (n=2178): smoked ≥100 TC, smoke sometimes or every day.	NA	**1364.95 (827.96)[c] ng/mL (U)**	**1250.35 (832.75)[b]** **1.092 (vs dual users)**	NA
Park et al., 2019, South Korea (45)	n = 15099 Mean age: 46.8, 49.5% females. Vapers (n=44): past month VP use. Dual users (n=246): smoked >100 TC, current smoking and past month VP use. Smokers (n=2627): smoked >100 TC and current smoking. Non-users (n=12182): smoked <100 TC, no current smoking and no past month VP use.	119.5 (53.9-49.2) ng/mL (U)	1030.5 (910.9-1165.7) 0.116	842.5 (792.2-896) 0.142	0.8 (0.2-0.8) 149.375

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Perez et al., 2021, US (47)	n = 1857, women between ages 18-49. % within age groups for vapers/smokers/non-users: 18-24: 16.1%/16.4%/22.9%, 25-34: 36.5%/35.6%/27.5%, 35-49: 47.4%/48%/49.6%. Vapers (n=109 for U analysis, n=74 for B analysis): self-reported VP use some or every day. Smokers (n=961 for U, n=536 for B): self-reported had smoked >100 TC, current some or everyday smoking. Non-users (n=787 for U, n=443 for B): self-reported never use of TC or VP.	**91.9 (34.7-243.2)**[c,d] **ng/mg creatinine (U)**	NA	**1507.6 (1067.5-2129.3)**[a,d] **0.061**	**0.4 (0.4-0.5)**[a,c] **229.75**
Piper et al., 2019, US (46)	n = 422 Mean (SD) age: 40.4 (14.1), 46.7% females, 63.7% White, 21.8% African American, 7.6% Multi-racial, 5.5% Hispanic. Dual users (n=256): smoking TC daily for >3 months and used VP at least once a week for the past 3 months. Smokers (n=166): smoking ≥5 TC per day for >6 months, no VP use in the past 3 months.	NA	**1209 (988) ng/mL (U)**	**1209 (802)** **1.0**	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Rostron et al., 2020, US (61)	See: Rostron et al., 2020	Open system: 563 (332.6-953) µg/g (U) Closed system: 118.8 (44.7-315) µg/g (U)	Open system: 291.7 (259-328.6) 1.930 Closed system: 310.2 (257-374.3) 0.383	NA	NA
Rudasingwa et al., 2021, South Korea (62)	See: Rudasingwa et al., 2021	**Median (IQR): 322.2 (0.9; 722.8)[c,d] ng/mL (U)**	NA	**729.5 (1185.8; 1342.6)[a,d]** **0.442**	**0.9 (0.9; 0.9)[a,c]** **358.0**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**7.5 (4.5-12.4) nmol/mg creatinine (U)**	**8.2 (4.6-14.8) (U)** **0.915**	**5.9 (3.8-9.3) (U)** **1.271**	**1.4 (0.6-3.5) (U)** **5.357**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Smith et al., 2019, US (64)	n = 211 Sociodemographic characteristics NR. Vapers, fruit flavour only (n=40): self-reported use of fruit flavoured VP in past 30 days. Vapers, tobacco flavour only (n=40): self-reported use of tobacco flavoured VP in past 30 days. Vapers, other flavour only (n=65): self-reported use of VP with other single flavour in past 30 days. Vapers, fruit and additional flavour (n=66): self-reported use of VP with fruit and an additional flavour in past 30 days.	**Fruit: 729.55 ng/mg creatinine (U)** **Tobacco: 1434.6** **Other: 854.7** **Fruit + other: 1256.2**	NA	NA	NA
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**2130 (1700–2669)**[c,d] **ng/mg creatinine (U)**	**1746 (1353–2253)**[d] **1.220**	**1376 (1103–1719)**[a,d] **1.548**	**1.0 (0.8–1.3)**[a,b,c] **2130.0**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Blood biosample					
Andersen et al., 2021, US (26)	n = 435 Vapers (n=35): VP use ≥once a week for the past year, smoking <100 TC in lifetime, no cannabis and other tobacco use for at least 1 year. Mean age: 23.5, 59.8% females. Smokers (n=112): ≥5 pack-years, current smoking of ≥2 TC per day, no VP use. Mean age: 41.2, 62.8% females. Non-users (n=269): smoking ≤100 TC or cannabis joints in lifetime, no cannabis and other tobacco products use in the past year, validated by serum cotinine levels <2 ng/mL. Mean age: 32, 69.7% females. Other (n=19): daily smokeless tobacco use, ≤100 TC in lifetime, no cannabis and other tobacco use for at least 1 year. Mean age 36.6, 5% females.	78 (45) ng/mL (BS)	NA	92 (34) 0.848	NR
Ghosh et al., 2018, US (40)	n = 41 Mean age: 29.3, 46.3% females, 53.7% white, 29.3% African American, 7.3% Asian, 7.3% Hispanic, 2.4% of other ethnicity, mean BMI: 28.5 kg/m^2. Vapers (n=10): <35 TC per week and >18 VP puffs per day. Smokers (n=13): >35 TC per week and <18 VP puffs per day. Non-users (n=18): never smoked or smoked <4 TC per week.	**97.2 (72.2) ng/mL (BS)**	NA	**140 (100.7)** **0.694**	**Below level of detection**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Ghosh et al., 2019, US (38)	See: Ghosh et al., 2019	**83.87 (75.46)[d] ng/mL (BS)**	NA	**148.33 (107.42)** **0.565**	**0.025 (0.06)[a]** **3354.8**
Jain 2021, US (59)	n = 11614 39.1% females, ethnicity within groups (White/Black/Hispanic/Other): Dual users: 49.4%/30.3%/14.1%/6.2%, Smokers: 48.9%/24.5%/20.6%/5.9%. Dual users (n=136): self-reported VP only or VP and TC use. Smokers (n=7977): self-reported TC use in past 5 days, BS cotinine levels ≥3.3 ng/mL.	NA	146.3 (116.2-184.2) ng/mL (BS)	152.5 (148.2-156.9) 0.959	NA
Rapp et al., 2020, US (60)	n = 428 Mean age: 42.0, 49.5% females, 71.7% non-Hispanic white. Vapers (n=49): VP use ≥1 time in past 30 days. Smokers (n=379): current smoking of TC every or some days and smoked ≥1 day in past 30 days.	**152.96 (33.66) ng/mL (BS)**	NA	**205.97 (10.47)** **0.743**	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Reidel et al., 2017, US (41)	n = 44 Mean age: 29.8, 61.4% females, 63.6% white, 27.3% African American, 11.4% Asian, 9.1% of other ethnicity, mean BMI: 27.0 kg/m^2. Vapers (n=15): self-reported VP use for ≥6 months and smoking <5 TC per week. Smokers (n=14): self-reported smokers. Non-users (n=15): self-reported non-users not regularly exposed to secondhand smoke.	**192.5 (66.32)[d] ng/mL (BS)**	NA	**183.9 (35.86)[d]** **1.047**	**0.06 (0.05)[a,c]** **3208.333**
Singh et al., 2019, US (42)	n = 48 Mean age: 34.6, 56.3% females, 60.4% white, 18.8% African American, 14.6% Asian, 6.3% Hispanic, mean BMI: 26.1 kg/m^2. Vapers (n=22): exclusive VP users. Non-users (n=26): never users of tobacco products.	**164.7 (39.92)[d] ng/mL (B)**	NA	NA	**3.86 (2.74)[a]** **42.668**
Hair biosample					
Clemens et al., 2019, US (33)	See: Clemens et al., 2019	NA	**0.153 (0.004-5.316)[d] pg/mg (H)**	**0.065 (0.009-0.465)[d]** **2.354**	**0 (0-0.001)[b,c]**
Doran et al., 2020, US (56)	See: Doran et al., 2020	35.8 (7.1-181.8) µg/g (H)	2523.1 (1944.2-3274.3) 0.014	NA	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Saliva biosample					
Chaffee et al., 2019, US (32)	n = 583, male schools' baseball team members Mean age: 15.8, 100% males, 49% non-Hispanic white, 40% Hispanic/Latino, 12% of other ethnicity. Vapers (n=12): only VP use in past 7 days. Dual users (n=16): VP and TC and/or smokeless tobacco use in past 7 days. Smokers (n=20): TC, cigars or hookah use in past 7 days. Non-users (n=467): no tobacco or VP use in past 7 days.	0.8 (0.61-1.03) ng/mL (S)	4.24 (1.53-11.7) 0.189	1.21 (0.63-2.33) 0.661	0.78 (0.74-0.82) 1.026
Mokeem et al., 2018, US (48)	n = 154 Mean age: 37.2, all males. Vapers (n=37): VP use for >12 months, never smoked TC. Smokers (n=39): smoking ≥5 CPD for past 12 months. Non-users (n=38): never used TC or VP.	**221.6 (29.4-252.4)[d] ng/mL (S)**	NA	**247.6 (227.6-263.4)[d]** **0.845**	**2.3 (1.7-3.2)[a,c]** **96.348**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Rubinstein et al., 2018, US (50)	n = 103, adolescents (13-18 years old). Mean age: 16.4, 33.7% females, 43.7% non-Hispanic white, 15.5% Asian American or Pacific Islander, 12.6% Multiracial, 26.2% Hispanic. Vapers (n=67): used VP ≥1 day in past 30 days and ≥10 lifetime use. Dual users (n=16): used VP and TC ≥1 day in past 30 days. Non-users (n=20): no use of VP and TC verified with undetectable cotinine and NNAL.	**Median (IQR): 0 (3.8)[b] ng/mL (S)**	**99.4 (139)[a,d]**	NA	**0 (0)[b]**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**179.6 (118.1-273.0) ng/mL (S)**	**149.2 (95.8-232.3) (S)** **1.204**	**174.8 (105.1-290.8) (S)** **1.027**	**83.9 (45.8-153.7) (S)** **2.141**
Wong et al., 2020, Malaysia (53)	n = 144 % within age groups: <25: 75%, ≥25: 25%, 2.1% females, 92.4% Malay, 5.6% Chinese, 2.1% Indian. Vapers (n=55): self-reported VP use. Dual users (n=89): self-reported VP and TC use.	**Median (IQR): 13.37 (3.5-97.7)[b] ng/mL (S)**	**94.3 (22.2-242.9)[a]** **0.142**	NA	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Ye et al., 2020, US (30)	n = 48 Mean age: 37.6, 50% females, 50% white, 22.9% African American, 12.5% Asian, 4.2% 'American Indian' descent, 10.4% of other race, 95.8% of non-Hispanic ethnicity. Vapers (n=12): NR. Dual users (n=12): NR. Smokers (n=12): NR. Non-users (n=12): NR.	**180.22 (272.42) ng/mL (S)**	**298.97 (432.67)**[d] **0.603**	**142.61 (174.11)** **1.264**	**0.56 (0.64)**[b] **321.821**

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Total nicotine equivalents (TNE)

Total nicotine equivalents include the molar sum of nicotine and its major metabolites cotinine and 3' hydroxycotinine and their glucuronide conjugates. Therefore, urinary TNE levels represent more than 90% of the nicotine dose and are not substantially affected by nicotine users' metabolic differences (72). Due to the combination of metabolites, TNE can better account for influences of nicotine metabolism and is considered the gold standard measure for nicotine intake (73).

RCTs

Two RCTs compared TNE levels in smokers who switched to ad libitum vaping product use for 7 days (8) and 8 weeks (5) (table 5). The McEwan and others study was conducted in confinement where participants could not use other than assigned nicotine products for the whole study period. Seven days after switching from smoking to using a prototype vaping product with 4.3mg/mL nicotine e-liquid, the study reported around 70% reduction in mean TNE levels compared with baseline, which was statistically significant. In comparison, average TNE levels in smokers who switched to non-use for 7 days had reduced by approximately 98%. Participants in the RCT by Hatsukami and others (5) were randomised to ad libitum use of a cartridge vaping product with 48mg/mL nicotine e-liquid, smoking or NRT (2 or 4 mg nicotine gum or lozenges) use for 8 weeks. The 7-day point prevalence smoking abstinence within vapers' group at the end of 8-week follow-up was 32.9% and among NRT users 17.1%. There were no statistically significant differences in average TNE levels within vapers who completely switched to vaping product use and other study groups at week 8.

Cross-over studies

No cross-over studies reported on TNE levels when using vaping products.

Longitudinal studies

TNE levels were also reported in 3 longitudinal studies that differed in follow-up lengths but were similar in reported results (18, 20, 23) (table 5): Jacob and others reported findings from 2 acute exposure studies—in one, participants were exposed to 15 puffs of a cartridge vaping product and in another study participants used their own vaping products ad libitum for 3 to 5 days (20). TNE levels 4 hours after the acute exposure and after ad libitum use of vaping products remained stable compared with baseline. Goniewicz and others reported no change in TNE levels after switching from smoking to vaping for 2 weeks (23), and TNE levels on average increased, but not statistically significantly, by 10% in Walele and others study 24 months after switching from smoking to use of a cartridge vaping product.

Table 5. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of total nicotine equivalents among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Urine biosample							
RCT							
Hatsukami et al., 2020, US (5)	8 weeks (S-M)	n=264 Smokers: smoking ≥5 TC a day Median age 47.0; 49.2% females; 53.5% white, 37.9% black, 8.7% other ethnicity. Vapers (n=76): cartridge VP (4.8% nicotine) ad lib use. Dual users (n=76): smoking and cartridge VP (4.8% nicotine) ad lib use. Smokers (n=36): own brand TC. NRT users (n=76): gum or lozenges (2 or 4 mg nicotine). Adherence: During the 8-week study period, median percent of smoke-free days within groups were: vapers=59.6%, dual users=0%, smokers=0%, NRT users=24.3%. CO-verified (<7 ppm) end-of-	n=58 60.1 (40.5) nmol/mg creatinine (U) ↓12.9%	n=65 55.6 (23.1) ↓11.5%	n=32 60.1 (44.4) ↓9.4%	NA	n=53 59 (39.8) ↓25.8%

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		treatment 7-day point prevalence abstinence within vapers group was 32.9%, within NRT users group was 17.1%.					
McEwan et al., 2021, UK (8)	7 days (A)	n = 148 Smokers: healthy smokers of non-menthol TC, 10-30 CPD for >3 years. Mean (SD) age: 35.9 (9.55), 41% females, 100% white. Vapers (n=30): ad lib use of prototype VP with 0.43% nicotine strength e-liquid of tobacco flavour. Smokers (n=30): ad lib use of TC (Lucky Strike, 0.63 mg nicotine, 7 mg tar). Non-users (n=29): abstained from using TC or VP for the study period. Other (n=30): ad lib use of HTP (glo). Adherence was enforced—the study was conducted in inpatient clinic without a possibility to use other products.	n=28 4.62[c,d] mg/24h (U) ↓**69.6%**	NA	n=30 14.88[a,d,e] ↓4.2%	n=29 0.39[a,c,e] ↓**97.6%**	n=28 7.37[c,d] ↓**48.4%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	n = 20 Smokers: smoking >5 TC per day for ≥12 past months. Mean (SD) age: 31 (9.7), 60% females, all Caucasian race. Vapers (n=9): at 2-week follow-up completely switched from smoking to cartridge VP (M201 Mild, 4.6 Volts, 280 mAh, 3.6-3.8 Ω) with 50/50 PG/VG ratio liquid with 11.0 mg/mL of nicotine. Dual users (n=11): at 2-week follow-up continued smoking TC and using the cartridge VP.	n=9 50 µmol/g creatinine (U) 0%	n=11 37 ↓26.0%	NA	NA	NA
Jacob et al., 2020, US (20)	4 hours after single use & 3-5 days (A)	Inpatient study, n = 13 46.2% females, 69.2% Caucasian, 15.4% Asian. Vapers: exclusive self-reported VP users. Outpatient study, n = 40 37.5% females, 57.5% Caucasian, 12.5% Asian. Vapers & dual users: use of VP >3 months. Vaping, inpatients (n=13): 15	Inpatients, n=11 56 (46) nmol/mg creatinine (U) ↓11.1% Outpatients, n=40	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		puffs every 30 seconds from own-brand VP (3 cartridge users, 8 tank and 2 modular). Vaping, outpatients (n=40): ad lib use of own-brand VP.	44 (34) ↓2.2%				
Walele et al., 2018, UK (18)	24 months (L)	n = 209 Smokers: self-reported smoking of 5 to 30 TC per day for ≥1 year. Mean (SD) age among those who switched: 38.7 (10.2), 44.1% females, mean (SD) BMI: 26.2 (4). Vapers (n=109): participants who switched to ad lib use cartridge VP (Puritane) with 1.6% nicotine strength, 67.5%/30% PG/VG vaping liquid with tobacco or menthol flavour.	n=102 Mean (SEM)=8.91 (0.65) mg/24h (U) ↑10.5%	NA	NA	NA	NA

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

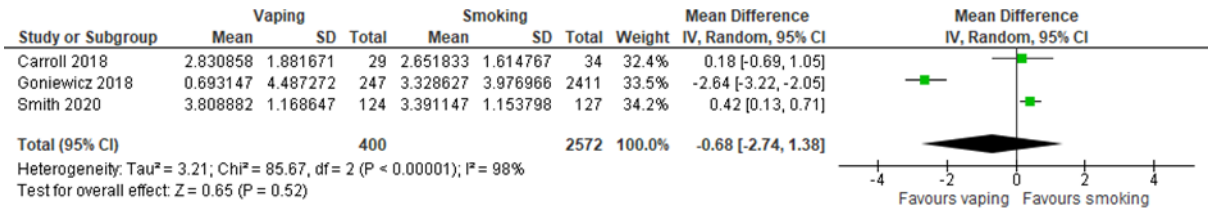
Four studies compared urinary TNE levels between vapers and smokers (table 6). Two found lower average levels of TNE for vapers, between 92% and 96% lower, with Goniewicz (36) reporting a statistically significant difference, whereas Coleman and others in their study of women of reproductive age did not test for statistical difference (54). Two studies observed higher average TNE levels among vapers compared to smokers, one statistically significantly higher of around 52% (52) with Carroll's study of adolescent boys reporting a non-statistically significant difference of around 20% (31).

Based on the largest study (36) that reported on TNE levels, vapers (some day or every day) had more than 92% lower levels compared with smokers (some day or every day). Coleman's study of women of reproductive age had used a similar definition of vaping and smoking (54). When more frequent vaping and smoking was taken into account, Smith and others (52) and Carroll and others (31) (among adolescent boys) reported higher levels of TNE among daily vapers compared to daily smokers.

Two studies compared levels of TNE across different vaping devices. Rostron and others reported that vapers who predominantly used tank type vaping products were exposed to higher levels of nicotine compared with those vapers who predominantly used cartridge or disposable vapes (61) although they did not test the differences statistically. Oliveri and others reported marginally higher levels of TNE among users of tank type vaping products compared to cartridge vaping products (44), but also did not test statistically.

Pooled across 3 studies (31, 36, 52), there were no statistically significant differences between average urinary TNE levels between vapers and smokers (figure 6). The geometric mean urinary TNE level was 49% lower among vapers than among smokers (LMD= -0.68, 95% CI -2.74, 1,38; p=0.52). There was substantial heterogeneity between the 3 studies (I^2= 98%).

Figure 6. Meta-analysis of cross-sectional studies reporting on urinary total nicotine equivalent levels between vapers and smokers



Three studies (36, 52, 55) assessed TNE among vapers versus non-users and all observed higher levels among vapers, 2 studies significantly so (36, 52). Dai and others found that vapers with self-reported respiratory symptoms had 230-fold higher levels of urinary cotinine than non-users with symptoms, but the study did not test for statistical

significance (55). In comparison, vapers without self-reported respiratory symptoms had 48% higher levels of urinary cotinine than non-users without symptoms, but again did not test for statistical significance.

Pooled across 2 studies (36, 52), average TNE levels were significantly higher among vapers than non-users (LMD=5.97, 95% CI 5.69, 6.25; p=0.0001, figure 7). The geometric mean urinary TNE level was 388 times higher among vapers than among non-users. There was little heterogeneity between studies (I^2= 0%).

Figure 7. Meta-analysis of cross-sectional studies reporting on urinary total nicotine equivalent levels between vapers and non-users

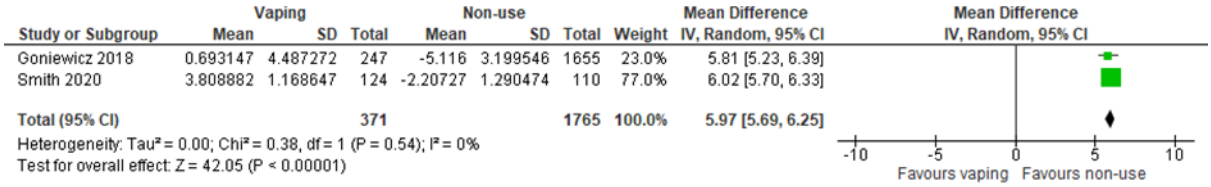


Table 6. Cross-sectional studies reporting on levels of total nicotine equivalents among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Urine biosample					
Carroll et al., 2018, US (31)	n = 94, of 'American Indian' descent. Median age within groups: vapers—33, dual users—39, smokers—45, 66% females. Vapers (n=29): everyday VP use in past 3 months and past 24 hours. Dual users (n=31): ≥5 CPD and everyday VP use in past 3 months, ≥1 TC in past 24 hours. Smokers (n=34): ≥5 CPD in past 3 months and past 24 hours.	**16.96 (8.55-33.64) nmol/mg (U)**	**15.79 (7.98-31.24)** **1.074**	**14.18 (8.24-24.40)** **1.196**	NA
Coleman et al., 2021, US (54)	n1 = 1504, non-pregnant women n2 = 109, pregnant women Vapers (n_1=111, n_2=7): self-reported VP use some days or everyday. Dual users (n1=370, n2=18): self-reported VP and TC use some days or everyday. Smokers (n1=1023, n2=84): self-reported smoking some days or everyday.	Non-pregnant: 0.44 (0.16-1.19) nmol/mg creatinine (U) Pregnant: 0.51 (0.08-3.49) nmol/mg creatinine (U)	11.62 (7.8-19.5) 0.038 24.88 (14.67-42.22) 0.020	12.33 (7.8-19.5) 0.036 25.08 (13.69-45.95) 0.020	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Dai et al., 2020, US (55)	See: Dai et al., 2020	Without symptoms: 2.3 (1.2-4.6) nmol/mg creatinine (U) With symptoms: 5.9 (2.3-14.7) nmol/mg creatinine (U)	12.5 (9.7-16.2) 0.184 31.1 (26.9-36.0) 0.190	NA	0.01 (0.01-0.01) 230.0 0.04 (0.03-0.06) 147.5
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**2.0 (1.1-3.5)[b,c,d] nmol/mg (U)**	**43.7 (39.8-48.1)[a,c]** **0.046**	**27.9 (23.8-32.7)[a,b]** **0.072**	**0.006 (0.005-0.007)[a]** **333.333**
Oliveri et al., 2020, US (44)	n = 217 Mean age: 40.0, 42.9% females, 55.3% white, 23.5% African American, 10.6% of other ethnicity. Vapers, tank VP (n=70) Vapers, cartridge VP (n=62) Smokers (n=62)	**Tank VP: 7 (7.7)[b] mg/g creatinine (U)** **Cartridge VP: 5.5 (6.8)[b] mg/g creatinine (U)**	**10.1 (6.3)[a]** **0.693 (vs tank VP)** **0.545 (vs cartridge VP)**	NA	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	**Dual users[b]**	**Smokers[c]**	**Non-users[d]**
Rostron et al., 2020, US (61)	See: Rostron et al., 2020	Open system: 8.8 (5.3-14.8) µmol/g (U) Closed system: 2 (0.7-5.4) µmol/g (U)	Open system: 35.3 (30.1-41.5) 0.249 Closed system: 40.1 (32.4-49.7) 0.050	NA	NA
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**45.1 (36.6–55.4)[c,d] ng/mg creatinine (U)**	**37.4 (29.6–47.2)[d]** **1.206**	**29.7 (24.2–36.3)[a,d]** **1.519**	**0.11 (0.09–0.14)[a,b,c]** **410.0**

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

3’-hydroxycotinine (3HC)

Cotinine is further metabolised to 3’ hydroxycotinine, and the ratio between the 2 nicotine metabolites is used to reflect the metabolism of nicotine.

RCTs and longitudinal studies

One RCT and one longitudinal study reported on 3-HC levels after 5 days (25) and 2 weeks use (23) (table 7). Liu and others study (the same RCT as (7)) reported statistically significant decrease in urinary and blood 3HC levels after completely switching from smoking to a cartridge vaping product use with 45mg/mL nicotine e-liquid for 5 days, while Goniewicz and others did not find significant changes in 3HC levels after 2 weeks of a cartridge vaping product use with 11mg/mL nicotine e-liquid (23).

Table 7. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of 3-hydroxycotinine among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Urine biosample							
RCT							
Liu et al., 2020, US (25)	5 days (A)	n = 153 Smokers: healthy smokers of ≥10 TC per day who smoked their first cigarettes of the day within 30 minutes after waking up. Vapers, original flavour (n=NR): ad lib use of cartridge VP (Vuse solo) with 4.5% nicotine strength and Original flavour liquid in confinement. Vapers, menthol flavour (n=NR): ad lib use of cartridge VP (Vuse solo) with 4.5% nicotine strength and Menthol flavour liquid in confinement. Other (n=NR): ad lib use of 4mg nicotine gum (Nicorette) in confinement.	Original flavour: % change from baseline (U): ↓**42.6%** Menthol flavour: % change from baseline (U): ↓**48.2%**	NA	NA	NA	NR

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 5571 µg/g creatinine (U) ↑7.7%	n=11 3962 ↓10.6%	NA	NA	NA
Blood biosample							
RCT							
Liu et al., 2020, US (25)	5 days (A)	See: Round et al., 2019.	Original flavour: % change from baseline (BP): ↓**29.3%** Menthol flavour: % change from baseline (BP): ↓**23.6%**	NA	NA	NA	NR

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

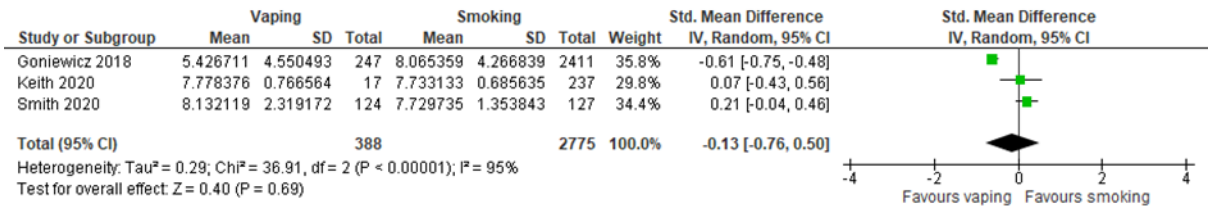
Eight studies reported urinary levels of 3-HC (table 8). One study reported data from the UK (51) and one pooled data from this UK study with data from the US and Poland (52). Five studies reported data from the US, 2 of which reported data from the Wave 1 PATH study (36, 47). Two further studies from the US (40, 58) reported levels of blood plasma or serum 3-HC.

Seven studies assessed differences in 3-HC between vapers and smokers. Four reported lower levels among vapers than smokers (36, 47, 49, 62); 2 of these studies reported statistically significant lower levels of around 93% (36, 47), the other 2 studies did not find statistically significant difference and reported 63% (62) and 28% (49) lower levels among vapers. Three studies reported higher levels among vapers (34, 51, 52). Smith and others reported a statistically significant 15-fold higher 3-HC levels among vapers, whereas Keith and others and Shahab and others reported 27% and 34% respectively higher levels among vapers than smokers, but the differences were not statistically significant.

Based on the largest sample size study (36), 3-HC was reportedly 93% lower among vapers compared to smokers. However, when more frequent vapers only were included, daily vapers had 15 times higher 3-HC levels than daily smokers (52).

Pooled across 3 studies (34, 36, 52), average 3-HC levels were lower among vapers than smokers (LMD= -0.13, 95% CI -0.76, 0.50, p=0.69; figure 8), but this difference was not statistically significant. Geometric mean urinary 3-HC levels were 12% lower among vapers than among smokers, and there was substantial heterogeneity between studies (I^2= 95%).

Figure 8. Meta-analysis of cross-sectional studies reporting on urinary 3-hydroxycotinine levels between vapers and smokers

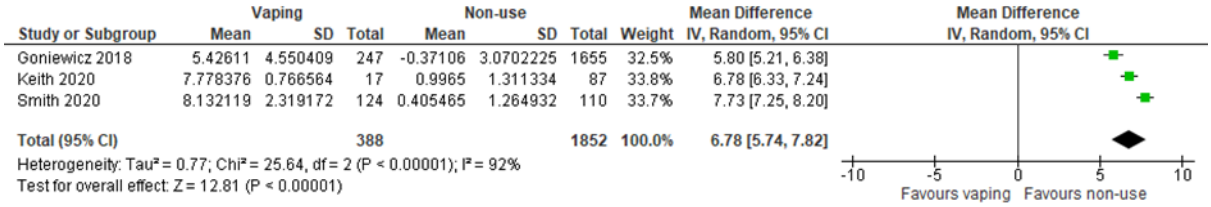


Two papers that measured blood 3-HC levels reported approximately 40% lower 3-HC levels among vapers than smokers (40, 58). Only Ghosh and others statistically tested the difference finding it not significantly different.

All studies that assessed urinary 3-HC levels among vapers and non-users reported significantly higher levels among vapers. Pooled across 3 studies (34, 36, 52), the geometric mean urinary 3-HC levels were 880 times higher among vapers than among

non-users (LMD= 6.78, 95% CI 5.74, 7.82, p=0.0001; figure 9). There was substantial heterogeneity between the 3 studies (I^2= 92%), but the direction of the difference was consistent across them.

Figure 9. Meta-analysis of cross-sectional studies reporting on urinary 3-hydroxycotinine levels between vapers and non-users



Only one study reported higher blood 3-HC levels among vapers than non-users but did not test for statistical differences (40).

Table 8. Cross-sectional studies reporting on levels of 3-hydroxycotinine among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Urine biosample					
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**227.4 (128.9-401.1)[b,c,d] ng/mg creatinine (U)**	**4985.7 (4533.5-5482.8)[a,c] 0.046**	**3182.3 (2682.7-3773.2)[a,b] 0.071**	**0.69 (0.6-0.8)[a] 329.565**
Keith et al., 2020, US (34)	See: Keith et al., 2020	**3204.1 (2865.3) ng/mg creatinine (U)**	**2527.8 (2196.4) 1.268**	**2887.6 (2237) 1.110**	**6.4 (13.7) 500.641**
Perez et al., 2021, US (47)	See: Perez et al., 2021	**181.5 (76.6-430.2)[c,d] ng/mg creatinine (U)**	NA	**2609.5 (1842.5-3695.7)[a,d] 0.070**	**0.7 (0.6-0.8)[a,c] 259.286**
Piper et al., 2019, US (46)	See: Piper et al., 2019	NA	**4937 (5378) ng/mL (U)**	**5495 (5624) 1.11**	NA
Rudasingwa et al., 2021, South Korea (62)	See: Rudasingwa et al., 2021	**Median (IQR): 820.3 (172.1; 2714.2)[d] ng/mL (U)**	NA	**2227.1 (500.3; 4802.3)[d] 0.368**	**2.6 (2.6; 2.6)[a,c] 315.5**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**11.4 (6.5-19.9) nmol/mg creatinine (U)**	**10.9 (6.0-19.8)** **1.046**	**8.5 (5.1-14.3)** **1.341**	**2.8 (1.2-6.3)** **4.071**
Shields et al., 2020, US (49)	n = 64 Mean age: 26.2, 78.1% females, 78.1% white, 7.8% Black, 7.8% Asian, 6.3% of other ethnicity. Vapers (n=13): use VP for >1 year and do not smoke for >5 months. Smokers (n=23): smoke >10 CPD for >6 months, do not use VP for >1 year. Non-users (n=28): smoked <100 TC, had not used TC or VP for >1 year.	**Median (IQR): 14 (0.8; 31.5)[d] nmol/mg (U)**	NA	**19.5 (5.8; 54.4)[d]** **0.718**	**0 (0; 0.1)[a,c]**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**34029 (3171–5117)[c,d] ng/mg creatinine (U)**	**23076 (2346–4034)[d]** **1.475**	**2275 (1798–2879)[a,d]** **14.958**	**1.5 (1.1–1.9)[a,b,c]** **22686.0**
Blood biosample					
Ghosh et al., 2018, US (40)	See: Ghosh et al., 2018	**26.1 (21.7) ng/mL (BP)**	NA	**43.3 (30.6)** **0.603**	0

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Jain, 2021, US (58)	n = 3264 39.4% females, 45.4% non-Hispanic white, 27.4% non-Hispanic black, 16.1% Hispanic; 5.2% non-Hispanic Asian. Vapers (n=98): VP use during the last 5 days. Dual users (n=116): VP and TC use during the last 5 days. Smokers (n=2285): smoking during the last 5 days.	32.52 (15.5-68.2) ng/mL (BS)	64.4 (51.1-81.7) 0.503	53.2 (10.2-56.3) 0.612	NA

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Summary of studies that reported on nicotine and nicotine metabolites

Across the 60 studies included in this section, only 5 (4 longitudinal and one cross-sectional) were from the UK. Across all studies, levels of nicotine and nicotine metabolites in participants using vaping products differed according to study design, definitions of vaping and smoking, biomarker and biosample used, and exposure length. Few meta-analyses could therefore be carried out. The maximum exposure length was 2 years, in only one of the longitudinal studies, most frequently studies were of acute, or short to medium exposure. From the quality of bias assessments, most studies had some methodological concerns.

Longitudinal studies generally indicated lower levels of nicotine exposure, although in some cases levels were similar to smokers. In the one meta-analysis that we were able to carry out for the longitudinal studies, 2 pooled cross-over studies found similar blood cotinine levels after a single use of a vaping product and smoking a tobacco cigarette. However, cross-over studies with longer 5- to 7-day conditions reported approximately 30% reduction in nicotine metabolites after vaping compared with smoking condition. RCTs with 5- and 7-day follow-ups reported statistically significant reductions in nicotine, cotinine and 3-HC levels after switching from smoking to ad libitum use of a vaping product. However, longitudinal studies with longer than a week follow-up did not find statistically significant changes in nicotine metabolites between vaping at follow-up and smoking at baseline. In addition to these findings, Dawkins and others and Soar and others studies (22, 24) suggest that vaping device characteristics (type and power), nicotine strength of e-liquid and experience in vaping (compensatory puffing) also affect how much nicotine users can derive from vaping products—with increasing exposure length smokers learn to derive similar levels of nicotine as they used to from smoking cigarettes.

There was substantial variation across the 39 cross-sectional studies included here, particularly in definitions of vaping and smoking, and length of exposure. We were able to carry out 4 meta-analyses for the different biomarkers (nicotine, cotinine, TNE and 3-HC) among at least weekly users, and each analysis indicated lower levels of nicotine biomarkers among vapers, but none were statistically significant. Two studies also examined nicotine exposure between tank type and disposable or cartridge vaping products, finding higher levels among the tank vaping products but differences were not tested significantly.

For comparisons between vapers and non-users, as expected, nicotine levels were statistically significantly higher among vapers. This was also consistent across the 4 meta-analyses carried out with the 4 different biomarkers.

As only 4 studies focused on adolescents, it is hard to discern any differential results from adults, and in general similar findings to adults were observed. The one longitudinal study that explored vaping product use among adolescents (19) reported an increase in average urinary cotinine levels after one year and an increase in the proportion of daily vapers, although there was wide variability in biomarker levels. The one study that examined participants with and without respiratory symptoms did not include exclusive smokers and did not carry out any statistical comparisons across any participant groups. As to be expected, urinary cotinine and TNE levels were higher among vapers with respiratory symptoms, than among non-users with respiratory symptoms.

Overall, evidence suggests that levels of nicotine and its metabolites differ widely between those who vape and those who smoke or dual use based on the vaping definition used in a study, frequency and length of vaping product use, and type of vaping products that are used. Findings from longitudinal studies reported little difference between vapers and smokers after medium- to long-term use, and pooled cross-sectional data of at least weekly vapers suggest that levels of nicotine and its metabolites are similar among smokers and vapers, and significantly higher in vapers than non-users.

7.4 Biomarkers of exposure to volatile organic compounds

The harm from tobacco smoking is primarily attributed to non-nicotine toxicants, such as volatile organic compounds (VOCs), a diverse group of chemicals formed by incomplete combustion of organic materials such as tobacco. It has been suggested that the thermal degradation of e-liquid constituents may result in exposure of VOCs in people who vape.

Study characteristics

The literature search identified 4 RCTs (5, 7, 8, 74), one crossover trial (75), 6 longitudinal studies (18, 21, 23, 24, 76, 77) and 13 cross-sectional studies (34-36, 44, 47, 50-52, 54, 55, 62, 64, 78) reporting on levels of biomarkers of volatile organic compounds (VOC). Many of these studies have also reported on nicotine metabolites and were presented in the prior narrative review. None of the included studies reported on the following volatile organic compounds: acetaldehyde, butyraldehyde, hydroquinone and propionaldehyde which are included in the WHO list of priority toxic contents and emissions of tobacco products (methods: table 3).

Of the 11 studies with more than one time-point (RCTs, cross-over and non-randomised longitudinal studies) reporting on biomarkers of VOCs, 6 were conducted in the US (5, 7, 21, 74, 75, 77), 4 were conducted in the UK (8, 18, 24, 76) and one in Poland (23). Three RCTs (7, 8, 74) and one longitudinal study (18) were funded by the tobacco industry (appendices: table 5).

Of the 13 cross-sectional studies, one study was conducted in the UK (51) and one pooled data from the UK, Poland and the US (52). One study was conducted in Italy (35), one in South Korea (62) and 9 in the US (34, 36, 44, 47, 50, 54, 55, 64, 78). Of those conducted in the US, 5 used data from Wave 1 of the PATH study (36, 47, 54, 55, 78) and one used data from Wave 2 of the PATH study (64). One cross-sectional study was funded by the tobacco industry (44).

Sample sizes of studies that reported on VOCs ranged from 20 in longitudinal studies by Dawkins and others and Goniewicz and others (23, 24) to 264 in an RCT by Hatsukami and others (5). Participants' age ranged from a mean of 30.1 (21) to a median of 47 years old (5), and between 22.2% (75) and 60% (23) were women. Most longitudinal studies explored VOC biomarker levels in participants from the general population except for Hickling and others (76) study from the UK, which explored the efficacy and acceptability of vaping products as harm reduction method in smoking patients with psychotic disorders.

Among cross-sectional studies, sample sizes ranged from between 67 (35) to 5211 (36), with women comprising between 2% (35) and 100% (47), and average ages ranging from 31.5 (34) to 40 (44) across general population samples. Five studies reported on a specific sample or a specific comparison. One focused on those working in an oil recycling facility (35), and one focused specifically on adolescents (50). One study focused specifically on comparisons between pregnant and non-pregnant women (54). One study compared people with and without respiratory symptoms (55). A further study compared those vaping cartridge products to those vaping tank products (44).

RCTs

The 4 RCTs (5, 7, 8, 74) recruited a total of 660 participants. Three RCTs funded by the tobacco industry (7, 8, 74) were conducted in confinement for 5 to 7 days and recruited smokers of at least 10 tobacco cigarettes per day, while an independent RCT by Hatsukami and others recruited smokers of at least 5 cigarettes per day that were followed for 8 weeks (5). Participants that were randomised to vaping groups were compared to groups that continued smoking, used NRT or HTP or did not use tobacco or nicotine products for the whole study period.

Cross-over studies

A single cross-over study (75) reported on VOC metabolite levels in 36 dual users who for one session used their own vaping products, smoked a tobacco cigarette or abstained from vaping or smoking for 3 days in confinement.

Longitudinal studies

Six longitudinal studies (18, 21, 23, 24, 76, 77) in total recruited 384 participants who were smokers or vapers. Levels of VOC metabolites were measured after a single use session

(77), one (24), 2 (23), 4 (21) and 6 weeks (76) after baseline, and 24 months after baseline (18). Comparison groups included vaping, concurrent vaping and smoking (after relapses from the vaping group) and one study included use of a tobacco pouch as a comparison group (77).

Cross-sectional studies

Two studies reported levels among daily vapers (52, 78), 4 among daily or some-day vapers (36, 47, 54, 55), 2 among at least weekly vapers (34, 51), 2 among at least monthly vapers (50, 64) and 2 did not define the frequency of participants' vaping (35, 44).

Non-use was also defined differently across studies. Two exclusively included participants who were ex-smokers, one grouped participants as non-user if they had not smoked for at least past 30 days (34), one if they had not smoked for at least 6 months (64) and one if they had not smoked for at least 6 months and were currently using NRT (51). One study included participants who were both ex- and never smokers (62). Four studies included only participants who had never smoked (47, 50, 78) and 2 studies did not define non-use (36, 55).

Risk of bias in included studies

RCTs

The 4 RCTs were assessed to have some concerns in overall risk of bias—the trials lacked information on the randomisation process and did not have or did not report pre-specified data analysis plans (appendices: table 1).

Cross-over studies

The cross-over study by St. Helen and others (75) was assessed to have some concerns in overall risk of bias, with concerns about randomisation, carry-over effects between trial conditions and lack of a pre-registered data analysis plan (appendices: table 2).

Longitudinal studies

Four longitudinal studies were assessed to be at moderate risk of bias (18, 21, 23, 24) and 2 at serious risks of bias (76, 77) (appendices: table 3). A key risk in studies at moderate risk of bias was associated with bias due to confounding because of participants' smoking, and 2 studies were judged to be at serious risk of bias because participants who used multiple nicotine products could participate in different study groups (77) and because of inconsistent reporting on study outcomes (76).

Cross-sectional studies

Quality of all cross-sectional studies was assessed using Biocross quality appraisal tool and is reported in appendices (appendices: table 4). Studies reporting levels of VOCs scored between 9 (50) and 16 (52) out of a maximum score of 20, with most studies of reasonable quality.

The main limitations were associated with lack of clear definition of smoking, vaping and non-use, as well as lack of discussion on limitations arising from the cross-sectional study design and limited detail about laboratory measurement procedures (blinded analyses, reporting on quality control procedures).

Study findings

Acrylamide (AAMA, GAMA, acrylamide equivalents)

Acrylamide is categorised as a probably carcinogenic compound to humans according to the IARC (79) and as having a potential carcinogenic effect on human health by the FDA (80). Tobacco is the main source of high levels of acrylamide exposure. However, acrylamide is also present in common foods, like potatoes, when cooked at high temperatures, so diet can also be a significant confounder in the exposure to acrylamide.

It is not yet clear how vaping product use might lead to increased acrylamide exposure (75). Two acrylamide metabolites that are used to measure exposure to this carcinogen are N-acetyl-S-(3-amino-3-oxopropyl)-cysteine (AAMA) and N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)-cysteine (GAMA).

RCTs

Two RCTs reported on urinary AAMA levels (5, 8); the McEwan and others trial also reported on urinary GAMA levels (table 9). Participants who switched from smoking to using a vaping product with 4.3mg/mL nicotine e liquid demonstrated declines in levels of both metabolites similar to participants not using nicotine products at all for 7 days—approximately 60% decline in AAMA and 35% in GAMA (8). Another RCT conducted in confinement (7) reported on a statistically significant approximate 50% reduction in levels of acrylamide equivalents after 5 days of switching from smoking to ad libitum use of a cartridge vaping product with 48mg/mL nicotine e-liquid. The acrylamide exposure reduction after switching to vaping was similar to reductions after switching to use of 4mg/mL nicotine gum (7). One RCT (5) followed participants for 8 weeks and reported an average 34% decline in AAMA levels compared with baseline smoking (of at least 5 cigarettes a day). However, this decline among vapers did not differ statistically significantly from changes in AAMA measured among dual users, smokers or NRT users (5).

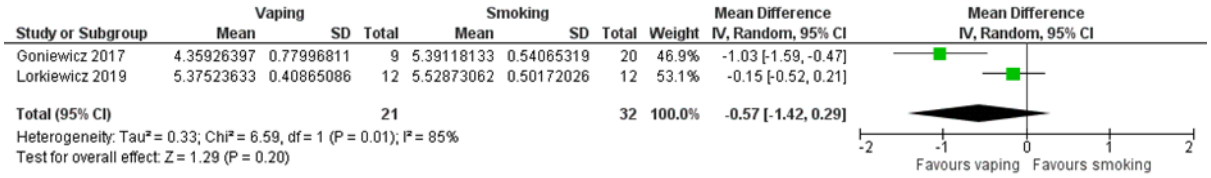
Cross-over studies

A single cross-over study (75) reported 70% higher AAMA levels after smoking a cigarette than after drawing 15 puffs from a cartridge or pod vaping product or 10 puffs from a tank vaping product (cigarette to vaping product GMR: 1.70, 95% CI: 1.50, 1.92) (table 9). The same study also reported 21% higher levels of AAMA after a vaping session compared with 3 days of enforced abstinence from nicotine or tobacco use (GMR: 1.21; 95% CI: 1.03, 1.43). Although authors noted that other sources could have contributed to acrylamide exposure, study findings were suggestive of increased acrylamide exposure after using a vaping product (75).

Longitudinal studies

Three longitudinal studies reported on AAMA levels after a single vaping product use (77), 2 (23) and 4 (21) weeks of vaping (table 9). Lorkiewicz and others also reported on GAMA levels after single use of a cartridge vaping product with 24mg/mL or 30mg/mL nicotine e-liquid. We pooled findings from Lorkiewicz and others and Goniewicz and others studies and found no evidence for different average urinary AAMA levels between smokers and vapers (LMD: -0.57, 95% CI: -1.42, 0.29; 53 participants, figure 10). However, there was considerable heterogeneity between the 2 studies at I^2 = 85%, they differed in exposure length (single use and vaping for 2 weeks) and in total included only 21 vapers.

Figure 10. Meta-analysis of longitudinal studies reporting on urinary AAMA levels (acrylamide) after exposure to vaping and smoking



Another longitudinal study which followed up 6 smokers who completely switched to ad libitum vaping of a tank vaping product for 4 weeks reported on average 50% reduction in AAMA levels compared with baseline (21).

Table 9. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of acrylamide among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
AAMA							
Urine biosample							
RCT							
Hatsukami et al., 2020, US (5)	8 weeks (S-M)	See: Hatsukami et al., 2020	n=58 354.6 (281.6-446.4) pmol/mg creatinine (U) ↓34.1%	n=64 408 (329.7-505) ↓26.9%	n=32 466.3 (363.6-598) ↑2.6%	NA	n=51 331 (255.9-428.8) ↓43.1%
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 0.07[c] mg/24h (U) **↓63.2%**	NA	n=30 0.18[a,d,e] **↓14.3%**	n=29 0.07[a,c,e] **↓65.0%**	n=28 0.12[c,d] **↓29.4%**
Cross-over							
St. Helen et al., 2020, US (75)	Single use (A)	n = 36 Dual users: ≥21 years old, smoking ≥5 TC per day over the past 30 days, use the same VP	n=36 122.9 (50.8)[c,d]	NA	n=36 190.2 (72.8)[a]	n=36 92.8 (37.2)[a]	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		with ≥6 mg/ml nicotine at least once daily for ≥15 days over the past 30 days. Mean (SD) age: 35.4 (11.7), 22.2% females, 61.1% white, 13.9% mixed, 11.1% Latino, 8.3% Black, 5,6% of Asian ethnicity. Vaping (n=36): 15 puffs from own-brand cartridge (33.3%) or pod VP (8.3%), or 10 puffs from own-brand tank VP (58.4%) with 30 seconds inter-puff interval. Nicotine strengths NR, 50% of vaping liquids were tobacco, 22.2% dessert candy, 13.9% fruit and 13.8% of menthol flavour. Smoking (n=36): smoking of own-brand TC. Non-use (n=36): enforced abstention from nicotine or tobacco use for 3 days in confinement. Served as reference for % change among vaping and smoking groups.	ng/mg creatinine (U) ↑32.4%		↑105.0%		

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 Mean=106 µg/g creatinine (U) ↓59.7%	n=11 113 ↓**54.1%**	NA	NA	NA
Lorkiewicz et al., 2019, US (77)	Single use (A)	n = 48 Smokers and/or vapers: self-reported smokers or vapers. Mean (SD) age: 34 (1), 35% females, 92% Caucasian, 6% NR their ethnicity, 2% African American. Vaping (n=12): after 48 hour washout, use of a cartridge VP (NJOY King) with 2.4% (tobacco flavoured) or 3% (menthol flavoured) nicotine strength vaping liquid. for <15 minutes and ≥15 puffs. Smoking (n=12): after 48 hour washout, smoking of a TC (Marlboro Red, 1.2 mg nicotine). Non-use (n=12): after 48 hour washout, on-use of nicotine or	n=12 234.8 (100.1) ng/mg creatinine (U)	NA	n=12 285.6 (152.8)	n=11 281.3 (311.2)	n=12 219.3 (139.2)

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		tobacco. Other (n=12): after 48 hour washout, use of tobacco pouches (Grizzly Premium Straight, ~10.5 mg/g nicotine) for <15 minutes.					
Pulvers et al., 2018, US (21)	4 weeks (S-M)	See: Pulvers et al., 2018	n=6 Mean (IQR) = 95.31 (69.6; 137.7) ng/mg creatinine (U) ↓50.4%	n=21 268.46 (168.6; 394.6)[e] ↑39.6%	NA	NA	n=10 96.52 (82.3; 157.3)[b] ↓49.8%
GAMA							
Urine biosample							
RCT							
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 18439.74[c] ng/24h (U) ↓**37.5%**	NA	n=30 33554.88[a, d,e] ↓8.0%	n=29 22522.66[a, c,e] ↓**38.4%**	n=28 24749.07[c, d] ↓**18.8%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Longitudinal							
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 1088.6 (2962.9) ng/mg creatinine (U)	NA	n=12 198.9 (133.7)	n=11 171.2 (174.9)	n=12 197.3 (163.9)
Acrylamide equivalents							
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=38: 53.1 (19.4) µg/24h (U) ↓**49.9%** Menthol, n=38: 49.7 (16.0) µg/24h (U) ↓**54.3%**	NA	NA	NA	Non-menthol, n=37: 49.7 (15.9) ↓**49.4%** Menthol, n=40: 55.9 (16.8) ↓**50.7%**

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

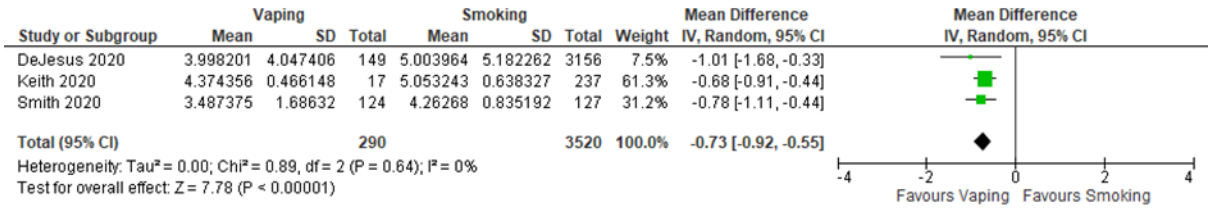
Cross-sectional studies

Eight studies reported on urinary levels of acrylamide (34-36, 47, 50-52, 78), 8 measured levels of AAMA and 6 measured levels of GAMA (table 10).

Seven studies compared levels of AAMA among smokers and vapers (34-36, 47, 51, 52, 78). All studies reported AAMA to be lower among vapers compared to smokers. Statistically significant differences were reported in 5 studies, findings levels of AAMA to be approximately between 47% (52) and 59% (36) lower among vapers compared to smokers. Frigerio and others and De Jesus and others also reported levels to be between around 51% and 63% lower among vapers compared to smokers, however these comparisons were not tested for statistical significance.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies were pooled to assess urinary AAMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary AAMA level was 52% lower among vapers compared to smokers (LMD= -0.73, 95% CI -0.99, -0.55; p<0.001; figure 11). There was no heterogeneity between the studies (I^2= 0%).

Figure 11. Meta-analysis of cross-sectional studies reporting on urinary AAMA between vapers and smokers

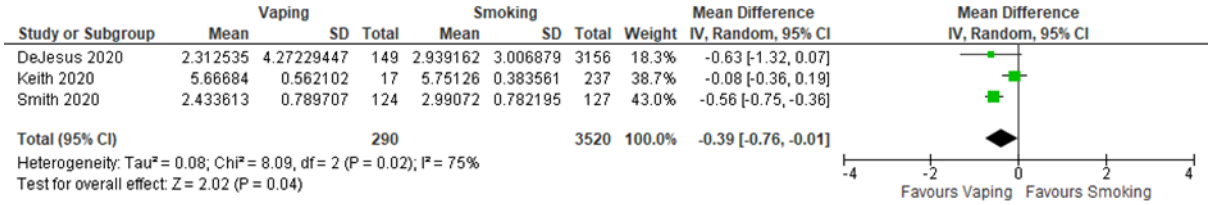


Six studies compared levels of GAMA among vapers and smokers (34-36, 51, 52, 78). All studies reported lower levels of GAMA among vapers compared to smokers. Levels were reported to be statistically significantly lower by around 43% to 46% among vapers compared to smokers in 3 studies (36, 51, 52). Keith and others and Frigerio and others reported levels to be between approximately 16% and 26% lower among vapers, however these comparisons were not statistically significant (34, 35). De Jesus and others reported levels to be on average 45% lower among vapers compared to smokers, this however was not tested for statistical significance (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), the same 3 studies were pooled to assess urinary GAMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary GAMA level was 32% lower among vapers compared to smokers (LMD= -0.39, 95% CI -0.76, -0.01; p=.040; figure 12). There was substantial heterogeneity between studies (I^2= 75%). Levels were reported among daily vapers and smokers by Smith and others (52) and De Jesus and others (78) and among at least weekly vapers by Keith and others (34). Smith and others (52) however used stricter

definition for vapers (daily use for 6 months and use of more than 5 cartridges, one bottle of e-liquid or 2 disposable vaping products a week and required a CO reading to bio-verify that vapers were not smoking). Keith and others sample of vapers was small (n=17).

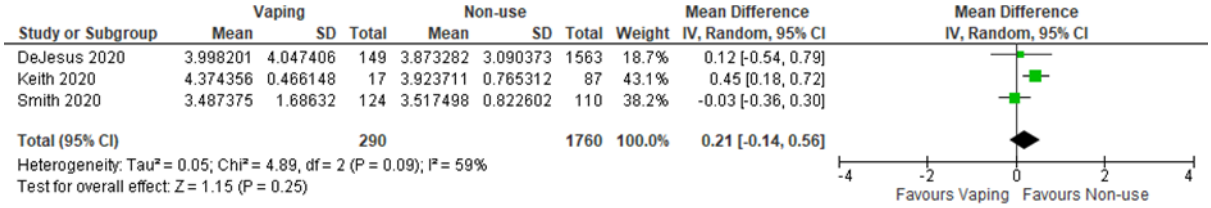
Figure 12. Meta-analysis of cross-sectional studies reporting on urinary GAMA levels between vapers and smokers



Eight studies compared levels of AAMA among vapers and non-users (34-36, 47, 50-52, 78). Levels of AAMA were reported to be around 31% statistically significantly higher among vapers in 2 adult samples (34, 47), and around 95% statistically significantly higher among vapers in an adolescent sample (50). Goniewicz and others and Smith and others reported levels to be between approximately 13% and 19% higher among vapers compared to non-users, however these differences were not statistically significant (36, 52). Levels of AAMA were also reported to be marginally higher—13% (78) to 17% (35)—by papers that did not test for statistical significance. Shahab and others reported that levels of AAMA were on average 12% lower among vapers compared to ex-smokers using NRT, however this was not statistically significant (51).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), the same 3 studies were once again pooled to assess urinary AAMA levels between vapers and non-users (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary AAMA level was 23% higher among vapers compared to non-users (LMD= 0.21, 95% CI -0.14, 0.56; p=0.25; figure 13). The difference was not statistically significant. There was substantial heterogeneity between studies (I^2= 59%). Although all studies reported levels among those who vape at least weekly, the definitions of non-use varied between studies. De Jesus and others (78) included those who self-reported never using tobacco, Keith and others (34) included those who had not smoked for at least 30 days, verified by a urinary cotinine level of below 10mg/mL. Finally, Smith and others (52) included those who had not smoked for at least 6 months, verified by expired air CO reading of 10 ppm or below.

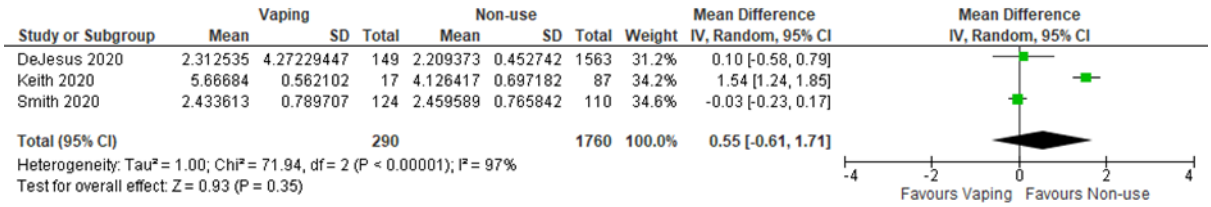
Figure 13. Meta-analysis of cross-sectional studies reporting on urinary AAMA levels between vapers and non-users



Six studies compared levels of GAMA among vapers and non-users (34-36, 51, 52, 78). Levels ranged from between around 16% lower (51) to 56% higher (35) among vapers compared to non-users, none of these differences however were reported to be statistically significant. De Jesus and others reported levels to be approximately 11% higher among vapers compared to non-user, this however was not tested for statistical significance (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), the same 3 studies were pooled to assess urinary GAMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary GAMA level was 73% higher among vapers compared to non-users (LMD= 0.55, 95% CI -0.61, 1.71; p=0.35; figure 14). The difference was not statistically significant. There was substantial heterogeneity between studies (I^2= 97%). As previously discussed, there was variation in the definitions of non-use between studies which might have increased heterogeneity between studies.

Figure 14. Meta-analysis of cross-sectional studies reporting on urinary GAMA between vapers and non-users



Across cross-sectional studies that measured urinary AAMA, vapers' levels were approximately between 37% and 53% and non-users' levels were approximately between 32% and 51% relative to AAMA levels detected among smokers. Across studies that reported urinary GAMA, vapers' levels were approximately between 54% and 84% and non-users' levels were between 47% and 65% of GAMA levels detected among smokers (figure 15).

Table 10. Cross-sectional studies reporting on levels of biomarkers of acrylamide among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
AAMA					
Urine biosample					
De Jesus et al., 2020, US (78)	n = 5,221 Demographic characteristics NR. Vapers (n=149): daily VP use. Smokers (n=3156): smoked >100 TC, current daily smoking. Non-users (n=1563): never used tobacco.	54.5 (4.69) ng/mL (U)	NA	149 (3.17) 0.366	48.1 (1.68) 1.133
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	Median (5th; 95th percentile): 55.8 (34.4-65.5) µg/g creatinine (U)	NA	114.6 (555.1-223.9) 0.487	47.9 (24.2-95.4) 1.165
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**56.05 (51.07-61.5)[c] ng/mg creatinine (U)**	**144.0 (136.4-151.9)** **0.389**	**136.4 (129.3-143.8)[a]** **0.411**	**47.28 (45.03-49.65)** **1.185**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Keith et al., 2020, US (34)	See: Keith et al., 2020	**88.5 (43.6)**[b,c,d] **ng/mg creatinine (U)**	**181.8 (157.4)**[a,d] **0.487**	**191.9 (136.1)**[a,d] **0.461**	**67.8 (60.5)**[a,b,c] **1.305**
Perez et al., 2021, US (47)	See: Perez et al., 2021	**58.8 (51.2-67.6)**[c,d] **ng/mg creatinine (U)**	NA	**135.1 (122.9-148.4)**[a,d] **0.435**	**44.9 (41.8-48.1)**[a,c] **1.310**
Rubinstein et al., 2018, US (50)	See: Rubinstein et al., 2018	**Median (IQR): 67.3 (69)**[b,d] **ng/mg creatinine (U)**	**235.6 (239.8)**[a] **0.286**	NA	**34.5 (41.6)**[a] **1.951**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**29.3 (22.3-38.3)**[b,c] **ng/mg creatinine (U)**	**82.4 (66.1-102.8)**[a,d] **0.356**	**65.6 (50.6-85.1)**[a,d] **0.447**	**33.6 (25.8-43.7)**[b,c] **0.872**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**37.9 (32.7–44.0)**[b,c] **ng/mg creatinine (U)**	**66.4 (56.2–78.4)**[a,d] **0.571**	**71.0 (61.4–82.1)**[a,d] **0.534**	**33.7 (28.8–39.3)**[b,c] **1.125**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
GAMA					
Urine biosample					
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	10.1 (0.53) ng/mL (U)	NA	18.4 (0.36) 0.549	9.11 (0.15) 1.109
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th percentile): 3.9 (1.4-6.7) µg/g creatinine (U)**	NA	**5.3 (1.7-30.4)[d]** **0.736**	**2.5 (0-7.1)[c]** **1.56**
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**9.924 (9.076-10.85)[c] ng/mg creatinine (U)**	**18.52 (17.57-19.52)** **0.536**	**17.33 (16.49-18.21)[a]** **0.573**	**9.022 (8.584-9.482)** **1.100**
Keith et al., 2020, US (34)	See: Keith et al., 2020	**36.5 (24.7) ng/mg creatinine (U)**	**39 (30.8)[d]** **0.936**	**43.6 (34.8)[d]** **0.837**	**25.4 (21.2)[b,c]** **1.437**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**10 (7.6-13.2)[b,c] ng/mg creatinine (U)**	**24.3 (19.6-30.2)[a,d]** **0.412**	**18.5 (14.7-23.3)[a,d]** **0.541**	**12.1 (9.5-15.5)[b,c]** **0.826**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**11.4 (9.9–13.1)[b,c] ng/mg creatinine (U)**	**18.7 (16.0–21.8)[a,d]** **0.610**	**19.9 (17.4–22.8)[a,d]** **0.573**	**11.7 (10.1–13.5)[b,c]** **0.974**

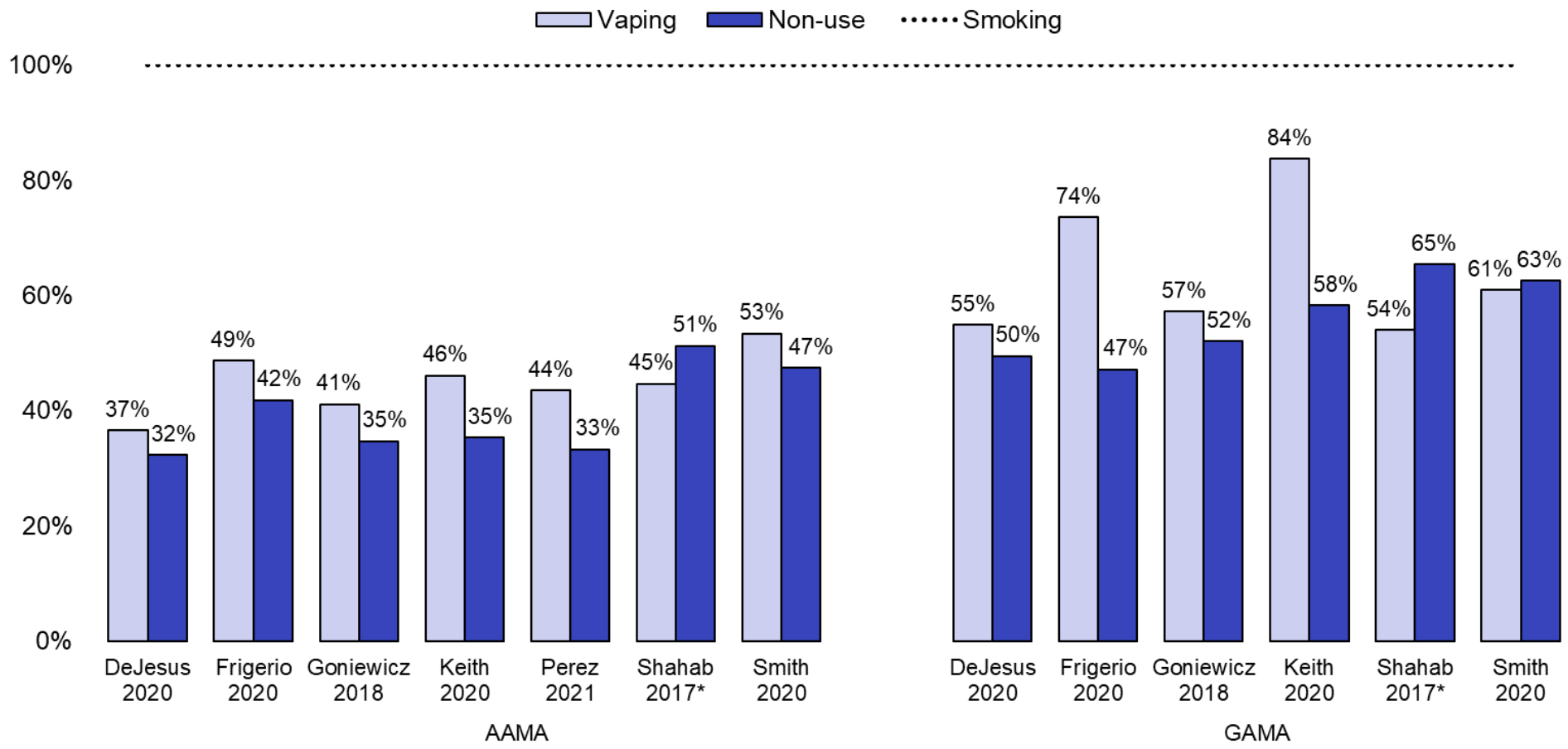
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 15. Levels of urinary acrylamide biomarkers in vapers and non-users relative to smokers



Note: * Non-users in Shahab et al. (51) were all using NRT.

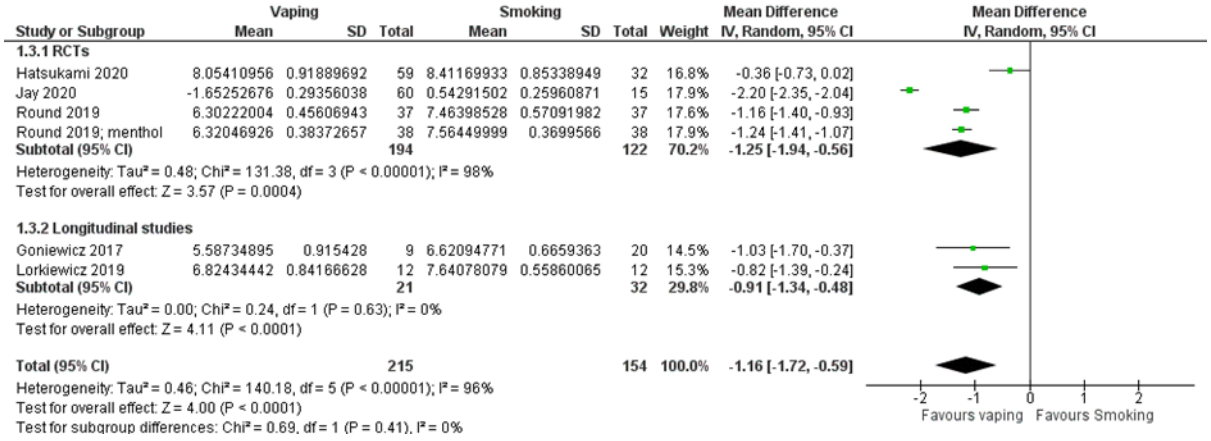
Acrolein (CEMA, 3-HPMA)

Acrolein is probably carcinogenic to humans according to the IARC (79) and is a severe respiratory and eye irritant (81, 82). Tobacco smoke is a major source of acrolein exposure, as is the combustion of fuels, wood, and plastics. It is also a common air pollutant generated in kitchens during roasting and frying at high temperatures (83). Acrolein is a thermal breakdown product of glycerine in e-liquids (81). The main urinary metabolites of acrolein are 3-hydroxypropylmercapturic acid (3-HPMA) and N-acetyl-S-(carboxyethyl)-l-cysteine (CEMA).

RCTs

Urinary levels of 3-HPMA were reported by 4 (5, 7, 8, 74) and urinary CEMA levels by 2 RCTs (5, 8) (table 11). We meta-analysed results from 3 RCTs on 3-HPMA levels between vapers and smokers (figure 16). Pooled across the 3 studies, average 3-HPMA levels were statistically significantly lower among vapers than smokers (LMD: -1.25, 95% CI: -1.94, -0.56; 316 participants); the geometric mean 3-HPMA level was 71% lower among vapers than among smokers (GMR: 0.29; 95% CI: 0.14, 0.57). Heterogeneity between the 3 RCTs was considerable at I^2 = 98%; 2 trials were conducted in confinement for 5 days (7, 74) and the third followed-up vapers for 8 weeks (5). The fourth RCT also reported statistically significant reductions in 3-HPMA and CEMA levels after 7 days of switching from smoking to vaping product or HTP use or non-use of tobacco and nicotine products (8).

Figure 16. Meta-analysis of RCTs and longitudinal studies reporting on urinary 3-HPMA levels (acrolein) after exposure to vaping and smoking



Cross-over studies

A single cross-over study (75) reported 270% higher 3-HPMA levels after smoking a cigarette than after drawing 15 puffs from a cartridge or pod vaping product or 10 puffs from a tank vaping product (cigarette to vaping product GMR: 3.70, 95% CI: 2.85, 4.79), which was in line with findings from RCTs (table 11). Compared with abstinence from

tobacco or nicotine products for 3 days, 3-HPMA levels were similar after single use of a vaping product (vaping product to abstinence GMR: 0.82, 95% CI: 0.67, 1.01).

Longitudinal studies

Six longitudinal studies (18, 21, 23, 24, 76, 77) reported levels of 3-HPMA and one (77) also reported levels of CEMA in vapers (table 11). Pooled data from 2 studies, one with single use (77), the other with 2-week exposure to vaping (23) also showed statistically significantly lower average levels of 3 HPMA in vapers than smokers (LMD: -0.91, 95% CI: -1.34, -0.48; 53 participants, I^2 = 0%). Lorkiewicz and others also concluded that a 48-hour tobacco cessation period was sufficient for smokers' 3-HPMA levels to reduce to the levels of non-tobacco users, that 3-HPMA levels reach maximum concentration approximately 40 minutes after smoking a cigarette and that acrolein metabolites are absent in the urine of participants after a single vaping product use session (77). Dawkins and others (24) reported no significant differences in urinary 3-HPMA level between vapers who used a tank vaping product for a week with different power settings (fixed and adjustable voltage) and different e-liquid nicotine concentration (6mg/mL and 18mg/mL). Hickling and others (76) found no significant changes in urinary 3-HPMA levels among a subset of participants (n=8) who were diagnosed with mental health disorders 6 weeks after they were encouraged to use vaping products instead of cigarettes. The 2 other longitudinal studies both reported a non-significant reduction in 3-HPMA levels in smokers who switched to vaping product use for 4 weeks (21) or 24 months (18), although Pulvers and others included only 6 participants who completely switched to vaping product use and Walele and others allowed non-adherence to the assigned vaping product in the vaping group.

Table 11. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of acrolein among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
CEMA							
Urine biosample							
RCT							
Hatsukami et al., 2020, US (5)	8 weeks (S-M)	See: Hatsukami et al., 2020	n=58 175.8 (116.8-264.7)[b] pmol/mg creatinine (U) ↓67.1%	n=65 337.5 (258.6-440.4)[a] ↓24.2%	n=32 368.4 (257.5-526.9) ↓3.2%	NA	n=53 251.4 (168.6-375.1) ↓55.6%
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 0.03[c] mg/24h (U) ↓**88.0%**	NA	n=30 0.24[a,d,e] ↓4.0%	n=29 0.03[c] ↓**88.9%**	n=28 0.03[c] ↓**88.0%**
Longitudinal							
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 531.7 (386.4)	NA	n=12 671.7 (571.6)	n=11 332.7 (287.4)	n=12 264.9 (198)

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			ng/mg creatinine (U)				
3-HPMA							
Urine biosample							
RCT							
Hatsukami et al., 2020, US (5)	8 weeks (S-M)	See: Hatsukami et al., 2020	n=59 3146.6 (2489-3978.2) pmol/mg creatinine (U) ↓47.1%	n=65 3889.7 (3132.7-4829.7) ↓24.2%	n=32 4499.4 (3347.6-6047.4) ↑13.7%	NA	n=53 3505. 8 (2667.9-4607) ↓40.0%
Jay et al., 2020, US (74)	5 days (A)	n = 90 Smokers: healthy, naïve to vaping, smoking ≥10 TC per day for ≥12 months, confirmed via urine cotinine ≥500 ng/mL and exhaled CO ≥12 ppm. Mean (SD) age: 39.1 (11.4), 38% females, 80% white, 14% African American, 3% other, 2% 'American Indian' descent or Alaska Native, mean (SD) BMI: 28 (5.2).	n=60 0.2 (0.06) mg/24h (U) ↓**89.3%**	NA	n=15 1.78 (0.47) ↑2.9%	n=11 0.19 (0.06) ↓**89.4%**	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		Vaping (n=60): ad lib use of pod VP (JUUL) in confinement with 5% nicotine salt solution (59 mg/ml) in different flavours: 1) Virginia tobacco (n=15) 2) Mint (n=15) 3) Mango (n=15) 4) Crème (n=15) Smoking (n=15): ad lib use of own-brand TC. Non-users (n=15): no use of TC or VP in confinement.					
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 0.19[c] mg/24h (U) ↓**84.6%**	NA	n=30 1.37[a,d,e] ↑5.4%	n=29 0.16 [c] ↓**87.8%**	n=28 0.27 [c] ↓**77.9%**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 605.6 (291.2) µg/24h (U) ↓**70.5%** Menthol,	NA	NA	NA	Non-menthol, n=38: 512.5 (192) ↓**72.0%** Menthol, n=40:

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			n=38: 598.3 (238.3) µg/24h (U) ↓**71.0%**				623.5 (274.9) ↓**68.6%**
Cross-over							
Helen et al., 2020, US (75)	Single use (A)	See: St. Helen et al., 2020	n=36 258.8 (195.2)[c] ng/mg creatinine (U) ↓7.5%	NA	n=36 965.7 (674.3)[a] ↑245.0%	n=36 279.9 (140)	NA
Longitudinal							
Dawkins et al., 2018, UK (24)	1 weeks (A)	See: Dawkins et al., 2018	1) 6mg/mL, fixed power, n=20 211.8 (133.1) ng/mg creatinine (U) 2) 18mg/mL, fixed power,	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			n=20 262.7 (202.5) 3) 6mg/mL, adjustable power, n=20 224.1 (343.4) 4) 18mg/mL, adjustable power, n=20 378.4 (467.4)				
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 Mean=406 µg/g creatinine (U) ↓52.1%	n=11 413 ↓**59.1%**	NA	NA	NA
Hickling et al., 2019, UK (76)	6 weeks (S-M)	n = 50 Smokers: with an established clinical diagnosis of	n = 8 Non-	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		schizophreniform, schizophrenia, schizoaffective disorder or bipolar disorder, or attending an early detection service in a high-risk state; daily smoking, confirmed via exhaled CO >5 ppm. Mean (SD) age: 39.0 (10.7), 24% females, 46% white, 42% black, 12% other ethnic group. Diagnosis: 54% schizophrenia, 20% schizoaffective disorder, 16% bipolar disorder, 6% unspecified psychosis, 4% delusional disorder. Vaping (n=50): ad lib use of disposable VP (NJOY) with tobacco-flavoured 4.5% nicotine e-liquid. Participants were given free VP for 6 weeks, were encouraged to replace smoking with VP as much as possible and were informed about where they could purchase VP after initial 6 weeks. Compliance: at 6 weeks, 37% had reduced CPD by ≥50% and 7% had stopped smoking. At 10	significant change compared with baseline (p=0.092).				

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
		weeks, 26% had reduced CPD by ≥50% and 5% had stopped smoking. At 24 weeks, 25% (10 out of 40) had reduced CPD by ≥50% and 2.5% had stopped smoking.					
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 1311 (1331)[c] ng/mg creatinine (U)	NA	n=12 2432.8 (1472.2)[a,d]	n=11 993.4 (619.7)	n=12 967.5 (398.7)
Pulvers et al., 2018, US (21)	4 weeks (S-M)	See: Pulvers et al., 2018	n=6 Median (IQR) = 390.35 (370.4; 513.8) pg/mg creatinine (U) ↓52.3%	n=21 1014.69 (662.2; 3346) ↑23.9%	NA	NA	n=10 370.34 (308; 518.2) ↓54.8%
Walele et al., 2018, UK (18)	24 months (L)	See: Walele et al., 2018	n=102 Mean (SEM)=114	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			0 (118) µg/24h (U) ↓9.5%				

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

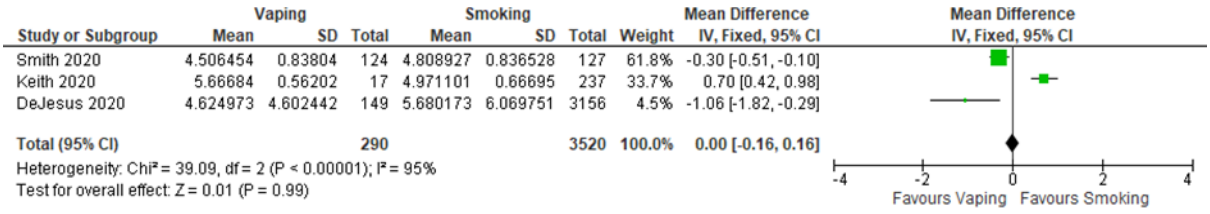
Cross-sectional studies

Thirteen studies reported on levels of acrolein (34-36, 44, 47, 50-52, 54, 55, 62, 64, 78), 10 reported levels of CEMA (34-36, 47, 51, 52, 55, 62, 64, 78), and 9 reported levels of 3-HPMA (34-36, 44, 50-52, 54, 78) (table 12).

Eight studies compared levels of CEMA between vapers and smokers (34-36, 47, 51, 52, 62, 78). Six studies reported levels to be statistically significantly different, between approximately 26% (52) and 98% (35) lower among vapers compared to smokers. De Jesus and others reported levels to be on average 75% lower among vapers, however this was not tested for statistical significance (78). Keith and others reported levels to be around 88% higher among vapers compared to smokers, however this was not statistically significant (34).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies were pooled to assess urinary CEMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary CEMA level as no different among vapers compared to smokers (LMD= 0.0, 95% CI -0.16, 0.16, p=0.99; figure 17). There was substantial heterogeneity between studies (I^2= 95%). As previously discussed, although all studies included those who vaped at least weekly, there was wide variation in the frequency of vaping between studies. Moreover, only one study by Smith and others (52) bio-verified smoking and vaping status with others relying on self-report.

Figure 17. Meta-analysis of cross-sectional studies reporting on urinary CEMA levels between vapers and smokers

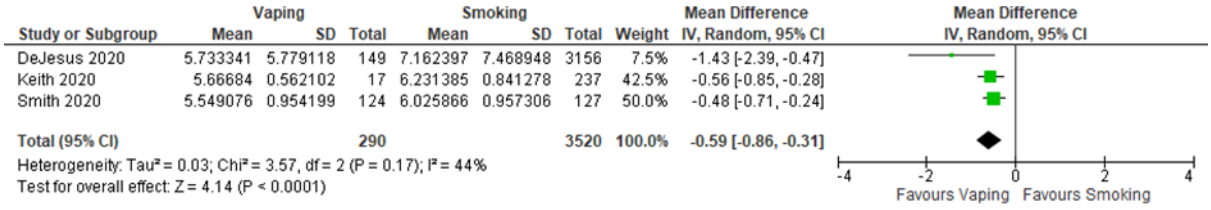


Seven studies compared levels of 3-HPMA (34-36, 51, 52, 54, 78). All 7 studies reported lower levels among vapers compared to smokers. Five studies reported levels were between 38% (52) and 83% (35) lower among vapers compared to smokers, with all comparisons reported to be statistically significant. De Jesus and others (78) reported levels to be approximately 76% lower among vapers, and Coleman and others (54) reported levels to be 78% lower among pregnant women who vape and 57% lower among non-pregnant women who vape when compared to participants who smoke; however, neither of these studies tested for statistical significance.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), the same 3 studies were pooled to assess urinary 3-HPMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary 3-HPMA level was 45% lower among vapers

compared to smokers (LMD= -0.59, 95% CI -0.86, -0.31, p<0.001; figure 18). There was moderate heterogeneity between studies (I^2= 44%).

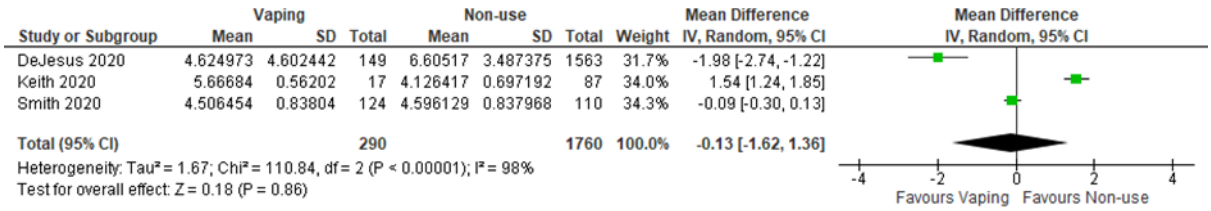
Figure 18. Meta-analysis of cross-sectional studies reporting on urinary 3-HPMA levels between vapers and smokers



Nine studies reported levels of CEMA between vapers and non-users (34-36, 47, 51, 52, 55, 62, 78). Three of these studies reported statistically significant differences, with levels on average between 1.13 times (13%) (47) and 4.29 times (329%) (34) higher among vapers compared to non-users. Four studies reported levels to be between approximately 10% higher (36) and 32% lower (62) among vapers than non-users, but none of these comparisons were statistically significant. De Jesus and others (78) reported that levels of CEMA were 2% higher among vapers than non-users, and Dai and others (55) reported urinary CEMA levels were 1% lower among vapers with respiratory symptoms compared with non-users with respiratory symptoms abut did not test for statistical significance.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), the same 3 studies were pooled to assess urinary CEMA between vapers and non-users (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary CEMA level was 12% lower among vapers compared to non-users (LMD= -0.13, 95% CI -1.62, 1.36; p=.86; figure 19). The difference was not statistically significant and there was substantial heterogeneity between studies (I^2= 98%), most likely due to variation in the definitions of non-use between studies.

Figure 19. Meta-analysis of cross-sectional studies reporting on urinary CEMA levels between vapers and non-users

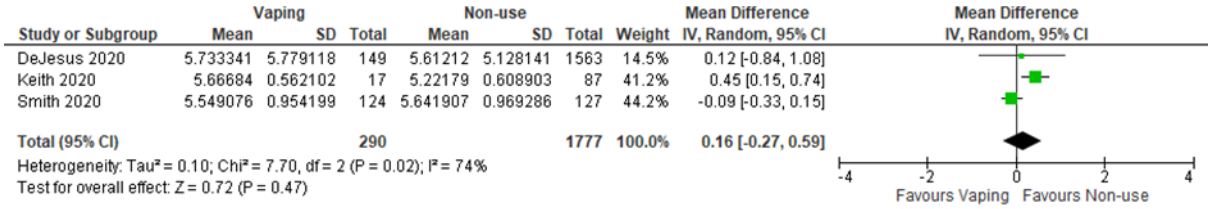


Seven studies reported levels of 3-HPMA between vapers and non-users (34-36, 50-52, 78). Rubenstein and others (50) reported statistically significantly higher urinary 3-HPMA levels (by approximately 32%) among adolescent vapers compared to adolescent non-users. Among adult samples, differences between 3-HPMA levels in vapers and non-users

ranged from 26% lower (51) to 52% higher (34) among vapers, however none of these comparisons were statistically significant. De Jesus and others reported levels to be 11% higher among vapers, however this was not tested for statistical significance (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies were pooled to assess urinary 3-HPMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary 3-HPMA level was 17% higher among vapers compared to non-users (LMD= 0.16, 95% CI -0.27, 0.59; p=.47; figure 20). The difference was not statistically significant. There was substantial heterogeneity between studies (I^2= 74%); as discussed, there was variation in the definitions of non-use between studies.

Figure 20. Meta-analysis of cross-sectional studies reporting on urinary 3-HPMA levels between vapers and non-users



Across cross-sectional studies that measured urinary biomarkers of acrolein, vapers' CEMA levels were approximately between 2% and 74%, and non-users' levels were approximately between 1% and 81% of CEMA levels among smokers. Across studies that measured urinary 3-HPMA, vapers' levels were approximately between 17% and 62%, and non-users' levels were between 12% and 68%, of 3-HPMA levels detected among smokers (figure 21).

Table 12. Cross-sectional studies reporting on levels of biomarkers of acrolein among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
CEMA					
Urine biosample					
Dai et al., 2020, US (55)	See: Dai et al., 2020	Without symptoms: 98.9 (90-108.8) ng/mg creatinine (U) With symptoms: 123.6 (102.3-149.2) ng/mg creatinine (U)	197.1 (180.4-215.4) 0.502 280 (261.7-299.5) 0.441	NA	99 (94.4-103.8) 0.999 103.9 (125.9) 1.190
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	102 (8.17) ng/mL (U)	NA	293 (7.7) 0.348	100 (3.27) 1.02

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th percentile): 2.7 (0.9; 36.5)[c,d] µg/g creatinine (U)**	NA	**163.1 (45.8; 358.4)[a,d] 0.017**	**0.9 (0; 2.1)[a,c] 3.0**
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**108.0 (95.93-121.6)[c] ng/mg creatinine (U)**	**302.0 (283.3-321.8)[c] 0.358**	**271.5 (255.1-289.0)[a,b] 0.398**	**98.14 (93.89-102.6) 1.100**
Keith et al., 2020, US (34)	See: Keith et al., 2020	**120.8 (90.3)[d] ng/mg creatinine (U)**	**188.1 (160)[d] 1.800**	**180.1 (134.8)[d] 1.880**	**79 (62.5)[a,b,c] 4.286**
Perez et al., 2021, US (47)	See: Perez et al., 2021	**98.7 (84.2-115.7)[c,d] ng/mg creatinine (U)**	NA	**235 (208.6-264.8)[a,d] 0.42**	**87 (81.1-93.3)[a,c] 1.134**
Rudasingwa et al., 2021, South Korea (62)	See: Rudasingwa et al., 2021	**Median (IQR): 11.9 (10; 92.7)[c] ng/mL (U)**	NA	**166.1 (25.3; 532.1)[a,d] 0.072**	**17.5 (10; 95.6)[c] 0.68**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**54.6 (41.7-71.4)[b,c] ng/mg creatinine (U)**	**141.8 (106.7-188.4)[a,d] 0.385**	**119.8 (88.2-162.9)[a,d] 0.456**	**67.8 (49.3-93.2)[b,c] 0.805**
Smith et al., 2019, US (64)	See: Smith et al., 2019	**Fruit: 95.5 ng/mg creatinine (U) Tobacco: 112.3 Other: 103.3 Fruit and other: 93.3**	NA	NA	NA
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**90.6 (78.1–105.0)[b,c] ng/mg creatinine (U)**	**126.9 (107.3–150.1)[a,d] 0.714**	**122.6 (105.9–141.8)[a] 0.739**	**99.1 (84.7–115.9)[b] 0.914**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
3-HPMA					
Urine biosample					
Coleman et al., 2021, US (54)	See: Coleman et al., 2021	Non-pregnant: 296.48 (207.92-422.67) ng/mg creatinine (U) Pregnant: 223.67 (157.69-317.25) ng/mg creatinine (U)	861.19 (726.78-1020.47) 0.344 1331.07 (974.09-1818.86) 0.168	697.75 (601.87-809.10) 0.425 1020.94 (783.61-1330.15) 0.219	NA
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	309 (26.5) ng/mL (U)	NA	1290 (31.2) 0.240	279 (10.1) 1.108
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th IQR): 222.1 (196.6; 738.2)[c] µg/g creatinine (U)**	NA	**1301.2 (328.9; 3661.1)[a,d]** **0.171**	**160.6 (77.9; 318.5)[c]** **1.383**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**314.8 (275.4-359.5)[c] ng/mg creatinine (U)**	**1317.8 (1225-1417.7)[c]** **0.239**	**1143.5 (1064.3-1228.6)[a,b]** **0.275**	**272.4 (257-288.6)** **1.156**
Keith et al., 2020, US (34)	See: Keith et al., 2020	**338.6 (206.4)[b,c] ng/mg creatinine (U)**	**569.5 (450.8)[a,d]** **0.595**	**724.4 (735.1)[a,d]** **0.467**	**223 (149.4)[b,c]** **1.518**
Oliveri et al., 2020, US (44)	See: Oliveri et al., 2020	**Tank VP: 899 (929.9)[b] µg/g creatinine (U)** **Cartridge VP: 852.3 (724.6)[b] µg/g creatinine (U)**	**1878.2 (1728.3)[a]** **0.479 (vs tank VP)** **0.454 (vs cartridge VP)**	NA	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Rubinstein et al., 2018, US (50)	See: Rubinstein et al., 2018	**Median (IQR): 254.3 (191.4)[b,d] ng/mg creatinine (U)**	**439.7 (224.1)[a]** **0.578**	NA	**192.8 (261.6)[a]** **1.319**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**175.3 (124-247.8)[b,c] ng/mg creatinine (U)**	**574.5 (429.1-769.2)[a,d]** **0.305**	**488.4 (345.1-691.2)[a,d]** **0.359**	**236.1 (168.1-331.6)[b,c]** **0.742**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**257 (216–304)[b,c] ng/mg creatinine (U)**	**405 (334–492)[a,d]** **0.635**	**414 (350–489)[a,d]** **0.621**	**282 (235–338)[b,c]** **0.911**

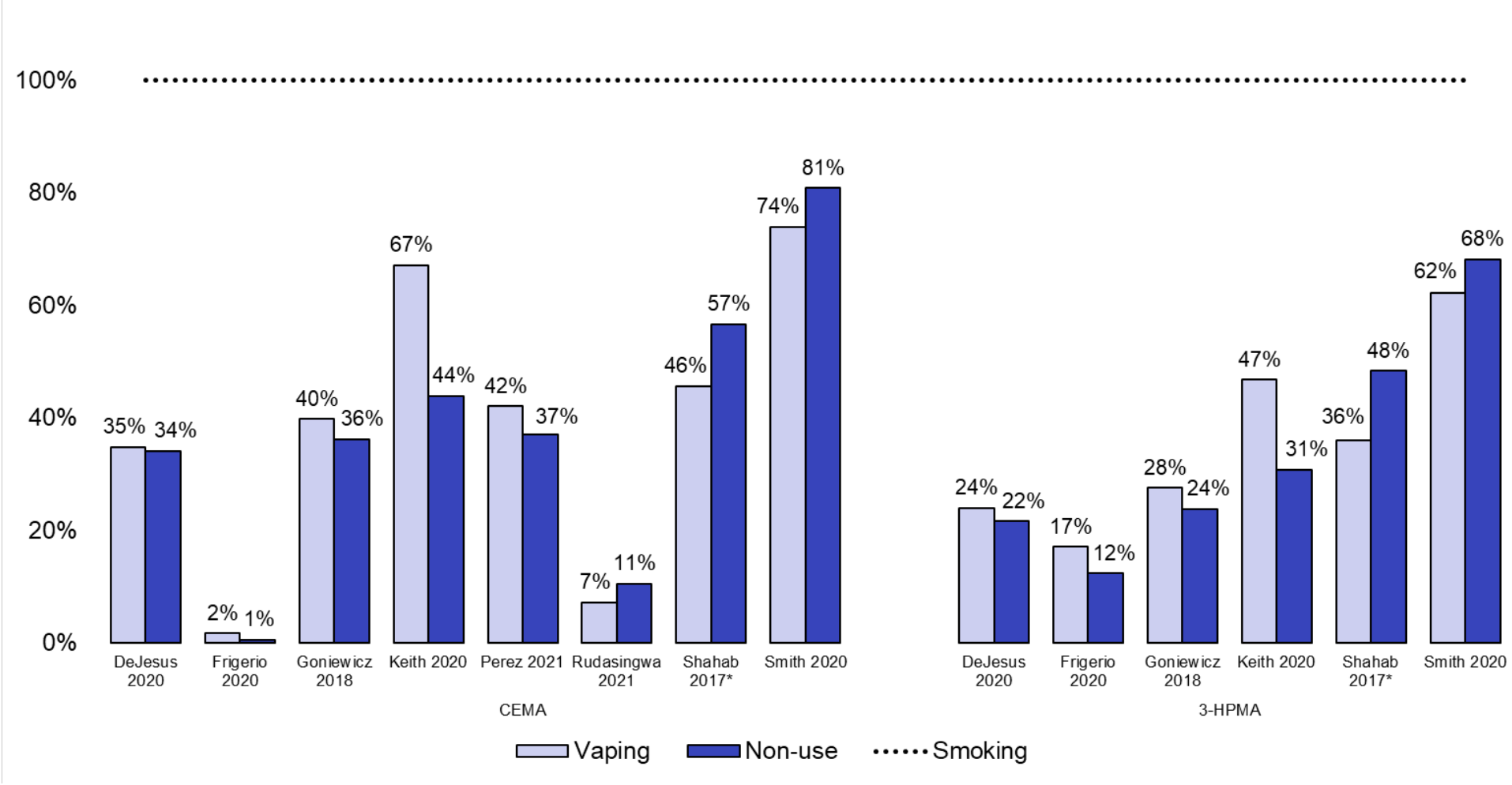
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 21. Levels of urinary acrolein biomarkers in vapers and non-users relative to smokers



Note: * Non-users in Shahab et al. (51) were all using NRT.

Acrylonitrile (CNEMA)

Acrylonitrile is considered possibly carcinogenic to humans according to the IARC (79) and is a carcinogen and respiratory toxicant to human health according to the FDA (80). Tobacco smoking is one of the main sources of non-occupational exposure. The main urinary metabolite of acrylonitrile is 2-cyanoethyl mercapturic acid (CNEMA).

RCTs

One RCT reported more than 80% reduction in levels of CNEMA in smokers who switched to ad libitum vaping product use in confinement for 5 days (7)—similar to the reduction in smokers who switched to using nicotine gum for the same period (table 13).

Cross-over studies

A single cross-over study (75) reported 609% higher CNEMA levels after smoking a cigarette than after drawing 15 puffs from a cartridge or pod vaping product or 10 puffs from a tank vaping product (cigarette to vaping product GMR: 7.09, 95% CI: 5.88, 8.54) (table 13). Compared with abstinence from tobacco or nicotine products for 3 days, CNEMA levels were approximately 36% lower after single use of a vaping product (vaping product to abstinence GMR: 0.64, 95% CI: 0.56, 0.74).

Longitudinal studies

Two longitudinal studies reported significant average reductions of around 90% in CNEMA levels after 2 and 4 weeks of switching from smoking to vaping product use (21, 23) (table 13). A single exposure study compared urinary CNEMA levels after vaping product use and smoking a cigarette with non-use of tobacco and nicotine products (77). Compared to non-use, it was concluded that urinary CNEMA levels were around 3 times higher after vaping product use and 4 times higher after smoking, suggesting that vaping might increase exposure to acrylonitrile in absolute terms (77).

Table 13. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of acrylonitrile among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
CNEMA							
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 36.8 (21.7) µg/24h (U) ↓**85.9%** Menthol, n=38: 36.5 (19.8) µg/24h (U) ↓**85.6%**	NA	NA	NA	Non-menthol, n=38: 29 (13.6) ↓**87.2%** Menthol, n=40: 34.7 (16.6) ↓**85.9%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Cross-over							
St. Helen et al., 2020, US (75)	Single use (A)	See: Helen et al., 2020	n=36 21.8 (19.7)[c,d] ng/mg creatinine (U) ↓33.7%	NA	n=36 140.9 (95.5)[a] ↑328.2%	n=36 32.9 (27.6)[a]	NA
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 Mean=24 µg/g creatinine (U) ↓89.4%	n=11 62 ↓**69.0%**	NA	NA	NA
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 65.7 (100)[d] ng/mg creatinine (U)	NA	n=12 91.1 (72.5)[d]	n=11 23.1 (75.8)[a,c]	n=12 11.4 (28.8)
Pulvers et al., 2018, US (21)	4 weeks (S-M)	See: Pulvers et al., 2018	n=6 Median	n=21 120.23	NA	NA	n=10 20.26 (8.4;

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			(IQR) = 4.82 (2; 7.9)[b] ng/mg creatinine (U) ↓94.6%	(51; 422.4)[a,e] ↑34.2%			32.7)[b] ↓77.4%

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

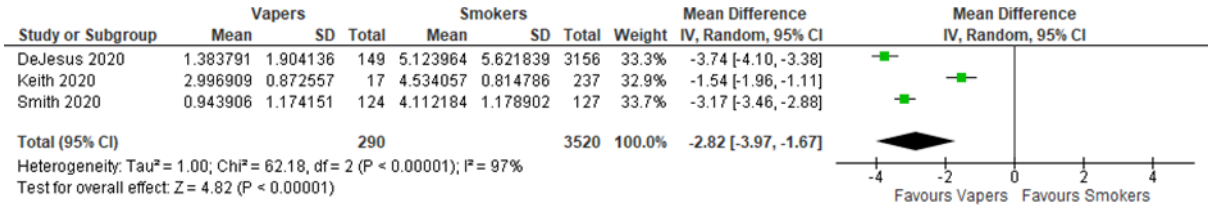
Cross-sectional studies

Eleven studies reported levels of acrylonitrile metabolite CNEMA (34-36, 47, 50-52, 54, 55, 62, 64, 78) (table 14).

Eight studies compared levels of urinary CNEMA among vapers and smokers (34-36, 47, 51, 52, 54, 62, 78). Six studies reported levels to be statistically significantly lower among vapers than smokers, by approximately 75% (34) to 99% (62). De Jesus and others reported levels to be 98% lower among vapers, and Coleman and others reported levels to be on average 88% lower among pregnant women who vape and 93% lower among non-pregnant women who vape when compared to smokers; however, neither study tested for statistical significance (54, 78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies were pooled to assess urinary CNEMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary CNEMA level was 94% lower among vapers compared to smokers (LMD= -2.82, 95% CI -3.97, -1.67, p<0.001; figure 22). There was substantial heterogeneity between studies (I^2= 97%). As previously discussed, although all studies included those who vaped at least weekly, there was wide variation in the frequency of vaping between studies. Moreover, only one study, Smith and others (52) bio-verified smoking and vaping status with other relying on self-report.

Figure 22. Meta-analysis of cross-sectional studies reporting on urinary CNEMA levels between vapers and smokers

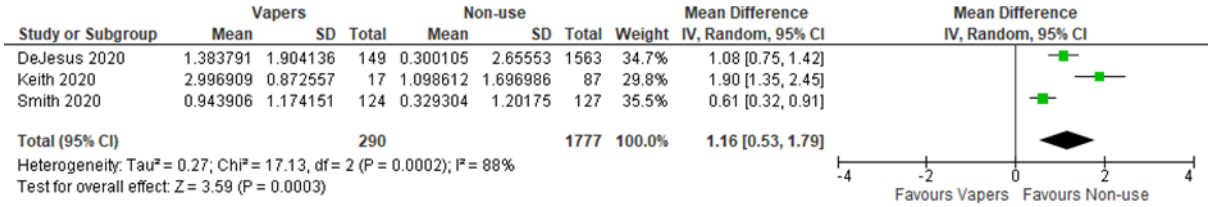


Eleven studies reported urinary levels of CNEMA among vapers and non-users (34-36, 47, 50-52, 55, 62, 78). Rubinstein and others reported statistically significantly higher levels among adolescent vapers compared to non-users (50). Among adults, 4 studies reported urinary CNEMA levels to be statistically significantly higher among vapers compared to non-users, by between around 85% (52) and 876% (34). Shahab and others reported levels to be approximately 98% lower among vapers compared to ex-smokers who use NRT, however these differences were not statistically significant (51). Rudasingwa and others reported no difference in urinary CNEMA levels between vapers and smokers (62). De Jesus and others reported levels to be on average 2.09 times (109%) higher among vapers and Dai and others reported that vapers with self-reported respiratory symptoms had approximately 213-fold higher levels of CNEMA than non-users with symptoms, and vapers without symptoms approximately 210-fold higher levels than non-users without

symptoms, but neither of these studies tested the comparisons for statistical significance (55, 78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), the same 3 studies were pooled to assess urinary CNEMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary CNEMA level was 3.19 times (219%) higher among vapers compared to non-users (LMD= 1.16, 95% CI 0.53, 1.79, p<0.001; figure 23). There was substantial heterogeneity between studies (I^2= 88%), which might be due to previously discussed variation in the definitions of non-use between studies.

Figure 23. Meta-analysis of cross-sectional studies reporting on urinary CNEMA levels between vapers and non-users



Across cross-sectional studies that measured urinary acrylonitrile metabolite CNEMA, vapers' levels were approximately between 0.2% and 22.6% and non-users' levels were approximately between 0.2% and 7.5% of CNEMA levels among smokers (figure 24).

Table 14. Cross-sectional studies reporting on levels of biomarkers of acrylonitrile among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
CNEMA					
Urine biosample					
Coleman et al., 2021, US (54)	See: Coleman et al., 2021	Non-pregnant: 4.36 (2.54-7.50) ng/mg creatinine (U) Pregnant: 8.48 (3.36-21.40) ng/mg creatinine (U)	82.05 (60.97-110.43) 0.053 96.74 (66.67-140.38) 0.088	63.07 (47.96-82.93) 0.069 73.98 (51.2-106.88) 0.115	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Dai et al., 2020, US (55)	See: Dai et al., 2020	Without symptoms: 3.4 (2.6-4.4) ng/mg creatinine (U) With symptoms: 7.3 (4.6-11.6) ng/mg creatinine (U)	58.1 (47.7-70.7) 0.059 122.5 (107.7-139.3) 0.060	NA	1.6 (1.5-1.8) 2.125 3.5 (3.0-4.1) 2.086
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	3.99 (0.55) ng/mL (U)	NA	168 (4.92) 0.024	1.35 (0.05) 2.956
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**3.959 (3.002-5.219)[c,d] ng/mg creatinine (U)**	**146.2 (133.8-159.8)[c]** **0.027**	**123.9 (109.9-139.7)[a,b]** **0.032**	**1.315 (1.23-1.406)[a]** **3.011**
Keith et al., 2020, US (34)	See: Keith et al., 2020	**29.3 (31.3)[b,c,d] ng/mg creatinine (U)**	**97 (78.6)[a,d]** **0.302**	**129.8 (126)[a,d]** **0.226**	**3.0 (12.3)[a,b,c]** **9.767**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Perez et al., 2021, US (47)	See: Perez et al., 2021	**3.8 (2.8-5.1)[c,d] ng/mg creatinine (U)**	NA	**97.1 (76.3-123.7)[a,d]** **0.039**	**1.2 (1.1.-1.3)[a,c]** **3.167**
Rubinstein et al., 2018, US (50)	See: Rubinstein et al., 2018	**Median (IQR): 1.3 (3.2)[b,d] ng/mg creatinine (U)**	**59.4 (81.3)[a]** **0.022**	NA	**0 (1.1)[a]**
Rudasingwa et al., 2021, South Korea (62)	See: Rudasingwa et al., 2021	**Median (IQR): 0.4 (0.4; 257.3)[c] ng/ml (U)**	NA	**179.9 (0.4; 592.4)[a,d]** **0.002**	**0.4 (0.4; 304.7)[c]** **0.002**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**1.4 (1.1-1.9)[b,c] ng/mg creatinine (U)**	**51.6 (33.6-79.2)[a,d]** **0.027**	**49.2 (32.9-73.6)[a,d]** **0.028**	**3.7 (2.1-6.5)[b,c]** **0.378**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Smith et al., 2019, US (64)	See: Smith et al., 2019	**Fruit: 7.55 ng/mg creatinine (U)** **Tobacco: 4.04** **Other: 2.79 (p<0.05 vs fruit flavour)** **Fruit and other: 4.56**			
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**2.57 (2.08–3.16)[b,c,d] ng/mg creatinine (U)**	**39.14 (30.91–49.57)[a,c,d]** **0.066**	**61.08 (49.74–74.98)[a,b,d]** **0.042**	**1.39 (1.12–1.74)[a,b,c]** **1.849**

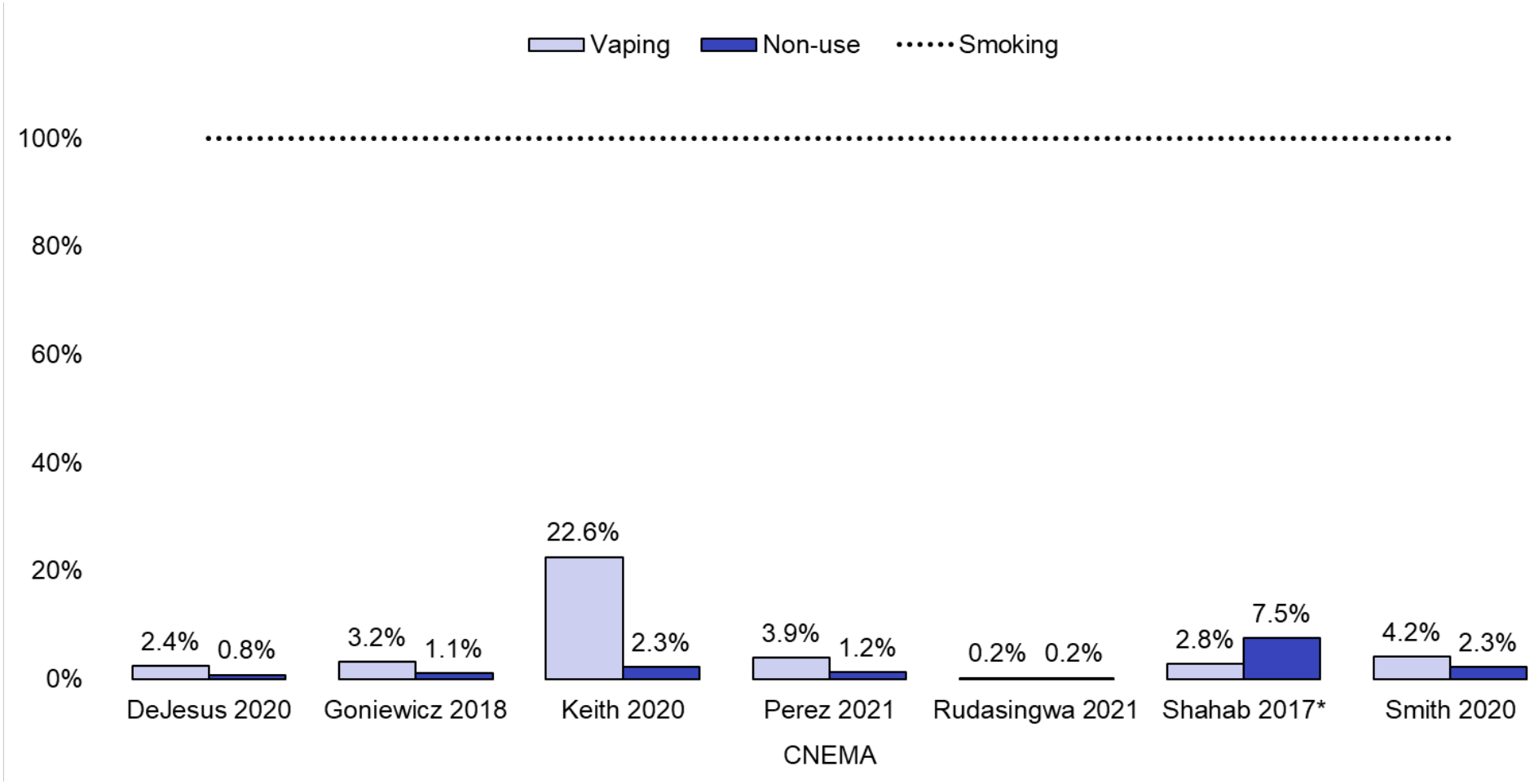
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another (p<0.05).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 24. Levels of urinary acrylonitrile biomarker CNEMA in vapers and non-users relative to smokers



Note: * Non-users in Shahab et al. (51) were all using NRT.

Benzene (S-PMA, MU)

Benzene is carcinogenic to humans according to the IARC (79) and is considered a carcinogen and cardiovascular, reproductive and developmental toxicant by the FDA (80). It is an abundant air pollutant arising from combustion and is a component of tobacco smoke and other air pollutants such as vehicle exhaust and industrial emissions (84). Depending on the temperature of the vaping product coil, benzene can be generated during vaping product use from thermal degradation of propylene glycol, vegetable glycerine and other additives, like benzoic acid and benzaldehyde (85). The main urinary metabolite of benzene is S-phenyl mercapturic acid (S-PMA), and some studies report levels of trans, trans-Muconic acid (MU) as a biomarker of benzene.

RCTs

Two RCTs reported change in urinary levels of S-PMA after switching from smoking to vaping product use in confinement for 5 (7) and 7 days (8) (table 15). Both studies reported average reductions of around 90% compared with baseline. These reductions were largest among a range of biomarkers measured in both RCTs, and changes in exposure to the metabolite of benzene were similar to reductions in non-users, nicotine gum users and HTP users.

Cross-over studies

A single cross-over study (75) reported 3.21 times (221%) higher S-PMA levels after smoking a cigarette than after drawing 15 puffs from a cartridge or pod vaping product or 10 puffs from a tank vaping product (cigarette to vaping product GMR: 3.21, 95% CI: 2.53, 4.07) (table 15). In comparison to abstinence from tobacco or nicotine products for 3 days, S PMA levels were 46% higher after single use of a vaping product (vaping product to abstinence GMR: 1.46, 95% CI: 1.13, 1.90).

Longitudinal studies

Three longitudinal studies reported on urinary S-PMA level changes among smokers who switched to vaping product use for 2 weeks (23), 4 weeks (21) and 24 months (18) (table 15). Levels of S-PMA statistically significantly decreased after 2 and 4 weeks of vaping (both studies report over 85% reduction), and a rapid decrease after product switching was also noted by Walele and others Pulvers and others also noted that urinary S-PMA levels were statistically significantly further reduced among smokers who completely switched to vaping compared with participants who were concurrently smoking and using vaping product for 4 weeks (21). Another longitudinal study measured urinary levels of the benzene metabolite MU after a vaping session, smoking a cigarette and using a nicotine pouch (77). Baseline average urinary MU levels were statistically significantly higher in 3 vapers than 12 non-users of tobacco products; after single use of a vaping product among vapers (n=12) and smoking a cigarette among smokers (n=12), average urinary levels of MU appeared to be lower in vapers—1700.4 versus 2018.2 ng/mg creatinine, respectively.

Table 15. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of benzene among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
(S)PMA							
Urine biosample							
RCT							
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 234.69[c] ng/24h (U) ↓**96.1%**	NA	n=30 5572.79[a,d,e] ↑9.1%	n=29 183.79[c] ↓**96.6%**	n=28 231.36[c] ↓**96.2%**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 0.4 (0.2) µg/24h (U) ↓**89.2%** Menthol, n=38: 0.4 (0.2) µg/24h (U) ↓**89.7%**	NA	NA	NA	Non-menthol, n=38: 0.4 (0.3) ↓**90.9%** Menthol, n=40: 0.5 (0.2) ↓**88.6%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Cross-over							
Helen et al., 2020, US (75)	Single use (A)	See: Helen et al., 2020	n=36 0.48 (0.31)[c,d] ng/mg creatinine (U) ↑14.3%	NA	n=36 1.77 (1.52)[a] ↑321.4%	n=36 0.42 (0.48)[a]	NA
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 Mean=77 ng/g creatinine (U) ↓**87.1%**	n=11 280 ↓70.6%	NA	NA	NA
Pulvers et al., 2018, US (21)	4 weeks (S-M)	See: Pulvers et al., 2018	n=6 Median (IQR) = 0.08 (0.07; 1) ng/mg creatinine (U) ↓88.7%	n=21 1.06 (0.6; 2.5)[e] ↑49.3%	NA	NA	n=10 0.09 (0.07; 0.6)[b] ↓87.3%

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Walele et al., 2018, UK (18)	24 months (L)	See: Walele et al., 2018	n=102 Mean (SEM)=2160 (302) ng/24h (U) ↓6.9%	NA	NA	NA	NA
MU							
Urine biosample							
Longitudinal							
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 1700.4 (743.3) ng/mg creatinine (U)	NA	n=12 2018.2 (3068.9)	n=11 1267.6 (874.7)	n=12 1472.5 (605.6)

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

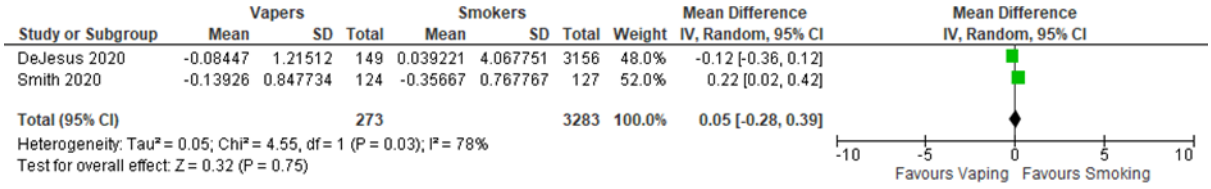
Cross-sectional studies

Eight studies reported levels of benzene (table 16). Seven reported on levels of the metabolite S-PMA (35, 36, 50-52, 64, 78) and 2 on MU (34, 78).

Five studies reported on levels of S-PMA among smokers and vapers (35, 36, 51, 52, 78). Frigerio and others reported levels to be significantly lower, by on average 67%, among vapers compared to smokers (35). Four other studies reported levels to be between around 12% lower (78) and 24% higher (52), among vapers compared to smokers, however none of these comparisons were statistically significant.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 2 studies were pooled to assess urinary S-PMA (36, 52). Combining the 2 studies, the pooled geometric mean urinary S-PMA level was 5% higher among vapers compared to smokers (LMD= 0.05, 95% CI -0.28, 0.39, p=0.075; figure 25). Differences were not statistically significant. There was substantial heterogeneity between studies (I^2= 78%). Smith and others (52) reported levels of bio-verified daily vapers and smokers, whereas Goniewicz and others (36) reported levels among those that self-reported daily or use on somedays of vaping products.

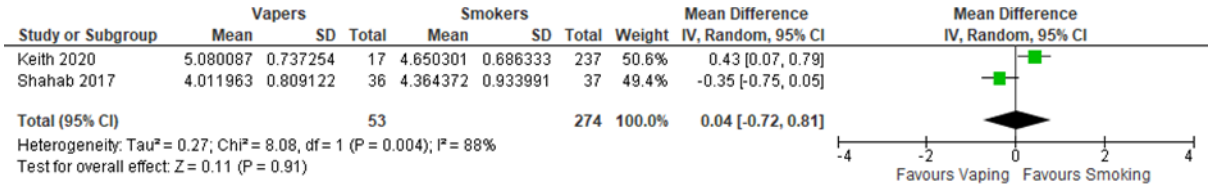
Figure 25. Meta-analysis of cross-sectional studies reporting on urinary S-PMA levels between vapers and smokers



Two studies reported levels of MU to be between approximately 59% higher (34) and 30% lower (51) among vapers compared to smokers, however both were not statistically significant.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 2 studies were pooled to assess urinary MU (34, 51). Combining the 2 studies, the pooled geometric mean urinary MU level was 4% higher among vapers compared to smokers (LMD= 0.04, 95% CI -0.72, 0.81; p=0.91; figure 26). This was not statistically significant. There was substantial heterogeneity between studies (I^2= 88%).

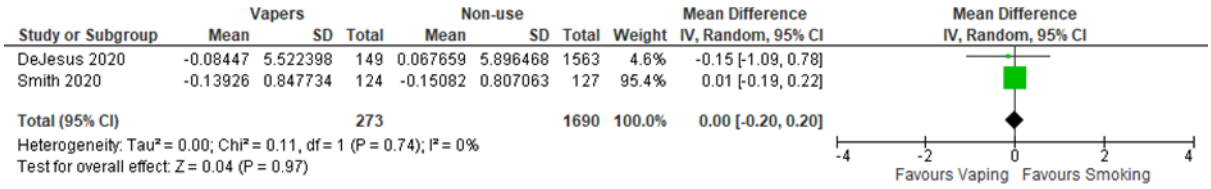
Figure 26. Meta-analysis of cross-sectional studies reporting on urinary MU levels between vapers and smokers



Six studies reported on levels of S-PMA among vapers and non-users (35, 36, 50-52, 78). Levels were reported to be no different among adolescent vapers and non-users (50). Among adult samples, levels were reported to be between approximately 14% lower (78) and 167% higher (35) among vapers compared to non-users, however these comparisons were not statistically significant.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 2 studies were pooled to assess urinary S-PMA. Combining the 2 studies, the pooled geometric mean urinary S-PMA level was the same among vapers as non-users (LMD= 0.00, 95% CI -0.20, 0.20; p=0.97; figure 27). The difference was not statistically significant. There was no heterogeneity between studies (I^2= 0%).

Figure 27. Meta-analysis of cross-sectional studies reporting on urinary S-PMA levels between vapers and non-users



Urinary MU levels were reported to be on average 52% higher among vapers compared to non-users (34), and on average 58% lower among vapers compared to ex-smokers who used NRT (51). Both comparisons were not statistically significant.

Across cross-sectional studies that measured urinary benzene biomarker S-PMA, vapers' levels were approximately between 33% and 124% and non-users' levels were approximately between 13% and 123% of S-PMA levels detected among smokers. Vapers' levels of biomarker MU were approximately between 70% and 159% and non-users' levels were between 105% and 168% of those reported among smokers (figure 28).

Table 16. Cross-sectional studies reporting on levels of biomarkers of benzene among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
(S)PMA					
Urine biosample					
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	0.919 (0.06) ng/mL (U)	NA	1.04 (0.024) 0.884	1.07 (0.042) 0.859
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th IQR): 0.16 (0.03; 0.34)[c] µg/g creatinine (U)**	NA	**0.48 (0.08; 1.45)[a,d]** **0.333**	**0.06 (0; 0.23)[c]** **2.667**
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**1.007 (0.9-1.125) ng/mg creatinine (U)**	**1.071 (1.017-1.127)** **0.940**	**1.090 (1.035-1.147)** **0.924**	**1.038 (0.967-1.114)** **0.970**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Rubinstein et al., 2018, US (50)	See: Rubinstein et al., 2018	**Median (IQR): 0 (0.1)[b] ng/mg creatinine (U)**	**0.2 (0.7)[a]**	NA	**0 (0)**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**0.74 (0.55-0.98)[b] ng/mg creatinine (U)**	**1.43 (1.11-1.83)[a,c]** **0.517**	**0.64 (0.48-0.84)[b]** **1.156**	**0.52 (0.37-0.71)** **1.423**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Smith et al., 2019, US (64)	See: Smith et al., 2019	**Fruit: 0.87 ng/mg creatinine (U)** **Tobacco: 0.98** **Other: 0.80** **Fruit and other: 0.89**	NA	NA	NA
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**0.87 (0.75–1.01) ng/mg creatinine (U)**	**0.92 (0.78–1.08)** **0.946**	**0.70 (0.60–0.80)** **1.243**	**0.86 (0.74–1.00)** **1.012**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
MU					
Urine biosample					
Keith et al., 2020, US (34)	See: Keith et al., 2020	**211 (179.3) ng/mg creatinine (U)**	**156.2 (147.6)** **1.351**	**132.4 (102.7)** **1.594**	**138.8 (92.3)** **1.520**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**55.2 (42.3-71.9)[b] ng/mg creatinine (U)**	**135 (102.3-178.1)[a]** **0.409**	**78.6 (58.2-106.2)** **0.702**	**131.8 (94.1-184.5)** **0.419**

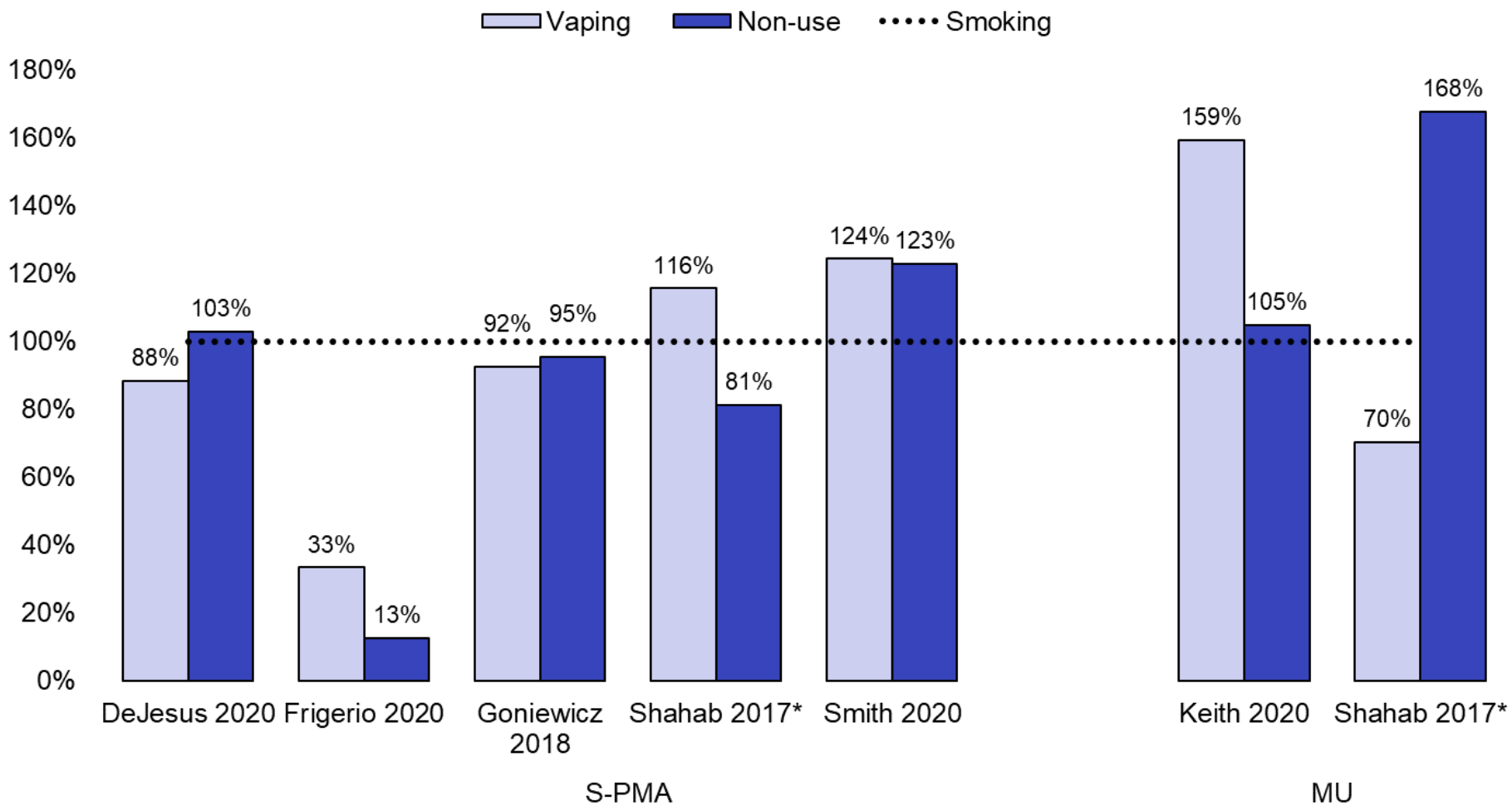
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 28. Levels of urinary benzene biomarkers in vapers and non-users relative to smokers



Note: * Non-users in Shahab et al. (51) were all using NRT.

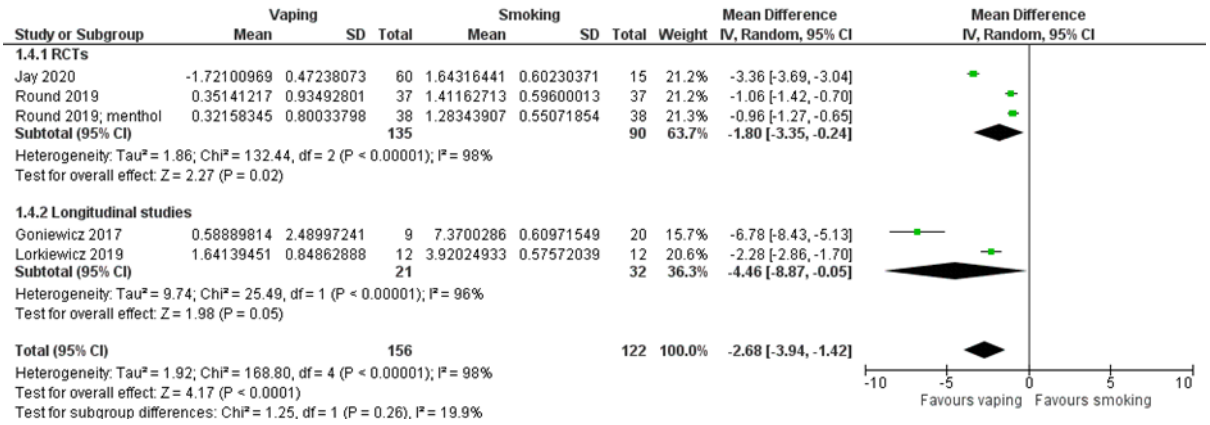
1,3-Butadiene (MHBMA, DHBMA)

1,3-Butadiene is carcinogenic to humans according to the IARC (79) and is a carcinogen, cardiovascular, reproductive and developmental toxicant according to the FDA (80). Tobacco smoke and automobile exhaust are 2 major environmental sources of 1,3-Butadiene (86). Monohydroxybutenyl mercapturic acid (MHBMA) and dihydroxybutylmercapturic acid (DHBMA) are 2 urinary biomarkers of exposure to 1,3-Butadiene. DHBMA is the most abundant and readily detected metabolite in humans while MHBMA is more sensitive; however, both have been used in population studies (86).

RCTs

Three RCTs funded by the tobacco industry and conducted in confinement for 5 (7, 74) and 7 (8) days reported on urinary MHBMA levels in smokers who switched to ad libitum vaping, nicotine gum use, HTP use or continued smoking (table 17). We pooled and meta-analysed data from 2 RCTs comparing vaping and smoking groups' exposure to 1,3Butadiene measured by urinary MHBMA levels (figure 29).

Figure 29. Meta-analysis of RCTs and longitudinal studies reporting on urinary MHBMA levels (1,3-Butadiene) after exposure to vaping and smoking



The average MHBMA levels were statistically significantly lower among vapers than smokers in 2 RCTs (LMD: -1.80, 95% CI: -3.35, -0.24; 225 participants); the geometric mean MHBMA levels were approximately 83% lower among vapers than among smokers (GMR: 0.17; 95% CI: 0.04, 0.79). Heterogeneity was high at I^2 = 98%, but the direction of the difference was consistent across both studies. Another RCT also reported a statistically significant reduction MHBMA levels of around 75% among smokers who completely switched to ad libitum vaping product use for 7 days (8).

Cross-over studies

A cross-over study by St. Helen and others (75) reported 5.8 times (480%) higher MHBMA levels after smoking a cigarette than after drawing 15 puffs from a cartridge or pod vaping product or 10 puffs from a tank vaping product (cigarette to vaping product GMR: 5.80,

95% CI: 3.73, 9.00) (table 17). In comparison to abstinence from tobacco or nicotine products for 3 days, MHBMA levels were 37% lower after single use of a vaping product (vaping product to abstinence GMR: 0.63, 95% CI: 0.48, 0.52).

Longitudinal studies

Two longitudinal studies reported on urinary MHBMA levels in vapers compared with smokers after a single use (77) and 2 weeks (23) after switching from smoking to vaping (table 17). Lorkiewicz and others also provided urinary DHBMA levels for non-users, vaping product users, smokers and tobacco pouch users. Pooled across the 2 studies, the average urinary MHBMA levels were statistically significantly lower among vapers than smokers (LMD: -4.46, 95% CI: -8.87, -0.05; 53 participants; figure 29); the geometric mean MHBMA levels were approximately 99% lower among vapers than among smokers (GMR: 0.011; 95% CI: 0.00014, 0.95). Heterogeneity between the 2 studies was high at I^2 = 96%, but the direction of the difference was consistent between studies, suggesting larger reduction in MHBMA levels after 2-week follow-up than after acute exposure. Lorkiewicz and others reported that levels of urinary DHBMA among vapers did not differ from levels among smokers, tobacco pouch users or non-users.

Table 17. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of 1,3-Butadiene among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
MHBMA							
Urine biosample							
RCT							
Jay et al., 2020, US (74)	5 days (A)	See: Jay et al., 2020	n=60 0.2 (0.1) µg/24h (U) ↓**96.3%**	NA	n=15 6.2 (4.1) ↑**14.8%**	n=11 0.2 (0.1) ↓**95.7%**	NA
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 684.97[c] ng/24h (U) ↓**77.5%**	NA	n=30 2552.74[a,d,e] ↓4.2%	n=29 123.17[c] ↓**96.1%**	n=28 240.28[c] ↓**90.4%**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 2.2 (2.6) µg/24h (U) ↓**55.1%** Menthol, n=38:	NA	NA	NA	Non-menthol, n=38: 1.9 (2) ↓**63.5%** Menthol, n=40:

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			1.9 (1.8) µg/24h (U) ↓**54.8%**				2.6 (2.5) ↓**38.1%**
Cross-over							
Helen et al., 2020, US (75)	Single use (A)	See: Helen et al., 2020	n=36 0.51 (0.42)[c,d] ng/mg creatinine (U) ↓27.1%	NA	n=36 3.43 (3.23)[a] ↑390.0%	n=36 0.70 (0.40)a	NA
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 Mean=40 ng/g creatinine (U) ↓**97.9%**	n=11 520 ↓**72.5%**	NA	NA	NA
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 7.4 (7.6)[c] ng/mg creatinine (U)	NA	n=12 59.5 (37.3)[a,d]	n=11 9.3 (11.9)[c]	n=12 6.2 (7)

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
DHBMA							
Urine biosample							
Longitudinal							
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 1577.5 (316.6) ng/mg creatinine (U)	NA	n=12 1535.2 (516)	n=11 1730.3 (900.7)	n=12 2015.2 (926.1)

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

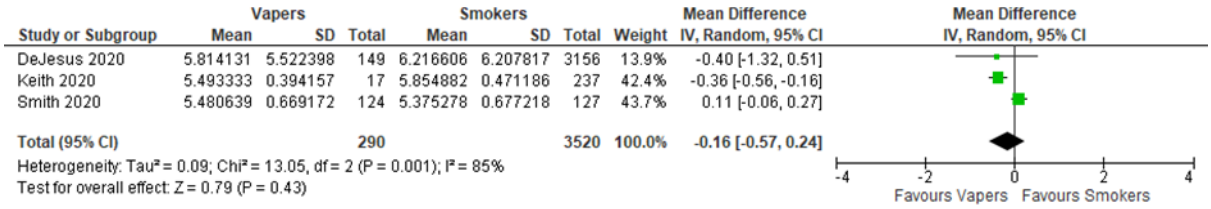
Cross-sectional studies

Seven studies reported on exposure levels to 1,3-Butadiene, 6 studies reported levels of urinary metabolite DHBMA (34-36, 51, 52, 78), and 7 reported levels of urinary metabolite MHBMA (34-36, 50-52, 78) (table 18).

Six studies reported levels of DHBMA among vapers and smokers (34-36, 51, 52, 78). Four studies reported levels to be statistically significantly lower among vapers compared to smokers, by between approximately 23% (51) and 45% (35). De Jesus and others reported levels to be on average 33% lower among vapers compared to smokers, however this was not tested for statistical significance (78). Smith and others reported levels to be statistically significantly higher, by 11%, among vapers compared to smokers (52).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies were pooled to assess urinary DHBMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary DHBMA level was 14% lower among vapers compared to smokers (LMD= -0.16, 95% CI -0.57, 0.24, p=0.430; figure 30). The difference was not statistically significant. There was substantial heterogeneity between studies (I^2= 85%). As previously discussed, although all studies included those who vaped at least weekly, there was wide variation in the frequency of vaping between studies. Moreover, only one study by Smith and others (87) bio-verified smoking and vaping status with others relying on self-report.

Figure 30. Meta-analysis of cross-sectional studies reporting on urinary DHBMA levels between vapers and smokers

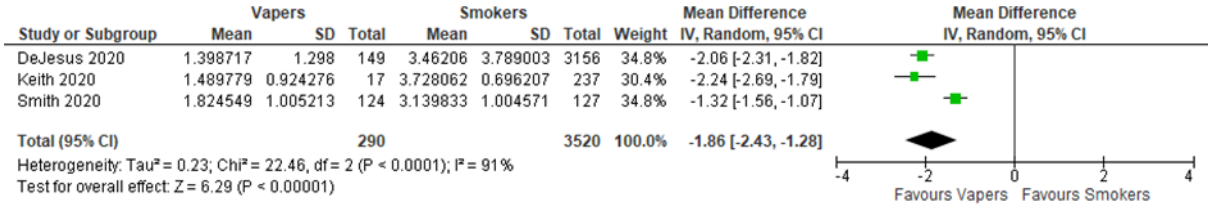


Six studies reported levels of MHBMA among vapers and smokers (34-36, 51, 52, 78). Five studies reported levels to be statistically significantly lower among vapers compared to smokers, with levels ranging between around 73% (52) to 86% lower (35) among vapers compared to smokers. De Jesus and others reported levels to be 87% lower among vapers, however this was not tested for statistical significance (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), the same 3 studies as above were pooled to assess urinary MHBMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary MHBMA level was 84% lower among vapers compared to smokers (LMD= -1.86, 95% CI -2.43, 1.28, p<0.001; figure 31). There was substantial heterogeneity between studies (I^2= 91%). As previously discussed, although all studies included those who vaped at least weekly, there was wide variation in

the frequency of vaping between studies. Moreover, only one study by Smith and others (88) bioverified smoking and vaping status with others relying on self-report.

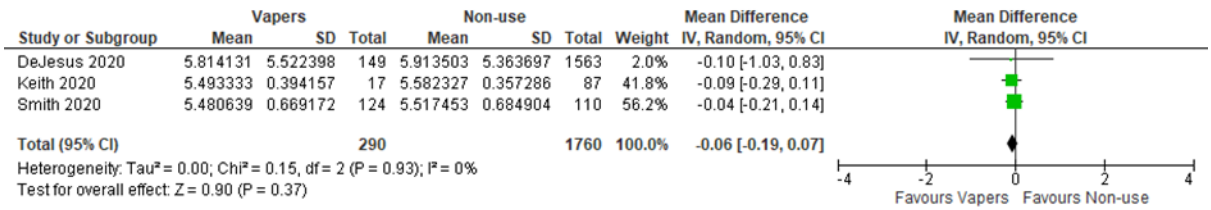
Figure 31. Meta-analysis of cross-sectional studies reporting on urinary MHBMA levels between vapers and smokers



Six studies reported levels of DHBMA among vapers and non-users (34-36, 51, 52, 78). Five studies found no statistically significant differences between vapers and non-users, with levels ranging between on average 25% lower (51) among vapers when compared to ex-smokers who use NRT, to 7% higher (35) among vapers compared to non-users. De Jesus found levels to be around 9% lower among vapers compared to non-users, however this was not tested for statistical significance (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies were pooled to assess urinary DHBMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary DHBMA level was 6% lower among vapers compared to non-users (LMD= -0.06, 95% CI -0.19, 0.07, p=0.37; figure 32). The difference was not statistically significant. There was no heterogeneity between studies (I^2= 0%).

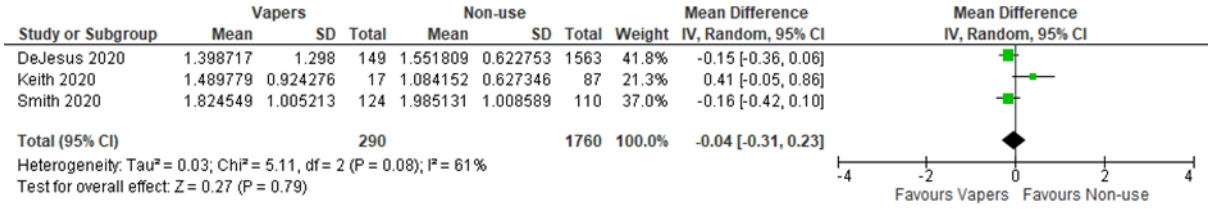
Figure 32. Meta-analysis of cross-sectional studies reporting on urinary DHBMA levels between vapers and non-users



Seven studies reported levels of MHBMA among vapers and non-users (34-36, 50-52, 78). Rubinstein and others reported a statistically significant difference between adolescent vapers and non-users, even though the groups reported the same median levels of MHBMA (50). Levels from studies among adults were reported to be between around 42% lower (51), when compared to ex-smokers who use NRT, to 104% higher (35) among vapers compared to non-users. Comparisons were not statistically significant. De Jesus and others reported levels to be 14% lower among vapers compared to non-users, however this was not tested for statistical significance (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), the same 3 studies were pooled to assess urinary MHBMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary MHBMA level was 4% lower among vapers compared to non-users (LMD= -0.04, 95%CI -0.31, 0.23, p=0.79; figure 33). The difference was not statistically significant. There was substantial heterogeneity between studies (I^2= 61%). As previously discussed, there was variation in the definitions of non-use between studies, which could have affected the heterogeneity between the 3 studies.

Figure 33. Meta-analysis of cross-sectional studies reporting on urinary MHBMA levels between vapers and non-users



Across cross-sectional studies that measured urinary DHBMA, vapers' levels were approximately between 56% and 111% and non-users' levels were approximately between 52% and 115% relative to urinary DHBMA levels detected among smokers. Across studies that reported urinary MHBMA, vapers' levels were approximately between 13% and 35% and non-users' levels were between 7% and 32% of levels detected among smokers (figure 34).

Table 18. Cross-sectional studies reporting on levels of biomarkers of 1,3-Butadiene among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
DHBMA					
Urine biosample					
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	335 (20.5) ng/mL (U)	NA	501 (8.84) 0.669	370 (11.1) 0.905
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th IQR): 263.8 (177.3; 298.7)[c] µg/g creatinine (U)**	NA	**479.1 (273.2; 925.6)[a,d]** **0.551**	**247.5 (163.6; 348.55)[c]** **1.066**
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**360.2 (340.9-380.4)[c] ng/mg creatinine (U)**	**532.7 (514.3-551.7)[c]** **0.676**	**499.8 (481.1-519.1)[a,b]** **0.721**	**359.0 (347.7-370.6)** **1.003**
Keith et al., 2020, US (34)	See: Keith et al., 2020	**262.7 (107.7)[b,c] ng/mg creatinine (U)**	**415.6 (209)[a,d]** **0.632**	**389.9 (194.4)[a,d]** **0.674**	**283.2 (104.5)[b,c]** **0.928**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**156.3 (126-193.8)[b,c] ng/mg creatinine (U)**	**294.9 (242.9-358)[a]** **0.530**	**202.7 (162.8-252.3)[a]** **0.771**	**204.2 (156.9-265.9)** **0.765**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**240 (212–270)[b,c] ng/mg creatinine (U)**	**251 (220–288)[a]** **0.956**	**216 (191–243)[a]** **1.111**	**249 (220–283)** **0.964**
MHBMA					
Urine biosample					
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	4.05 (0.3) ng/mL (U)	NA	31.9 (0.787) 0.127	4.72 (0.155) 0.858
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th IQR): 0.55 (0.14; 2.07)[c] µg/g creatinine (U)**	NA	**4.07 (0.74; 11.38)[a,d]** **0.135**	**0.27 (0; 2.47)[c]** **2.037**
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**4.308 (3.843-4.829)[c] ng/mg creatinine (U)**	**31.92 (29.64-34.38)[c]** **0.135**	**27.90 (26.04-29.89)[a,b]** **0.154**	**4.543 (4.348-4.745)** **0.948**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Keith et al., 2020, US (34)	See: Keith et al., 2020	**6.8 (7.9)[b,c] ng/mg creatinine (U)**	**18.7 (20.7)[a,d]**	**19.5 (15.4)[a,d]**	**3.6 (2.5)[b,c]**
Rubinstein et al., 2018, US (50)	See: Rubinstein et al., 2018	**Median (IQR): 0 (0)[d] ng/mg creatinine (U)**	**0 (0.1)**	NA	**0 (0.5)[a]**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**4.44 (3.42-5.78)[b,c] ng/mg creatinine (U)**	**36.6 (25.4-52.6)[a,d]** **0.121**	**29.8 (19.9-44.8)[a,d]** **0.149**	**7.67 (5.08-11.6)[b,c]** **0.579**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**6.20 (5.18–7.40)[b,c] ng/mg creatinine (U)**	**21.17 (17.31–25.88) [a,d]** **0.293**	**23.10 (19.40–27.51)[a,d]** **0.268**	**7.28 (6.03–8.79)[b,c]** **0.852**

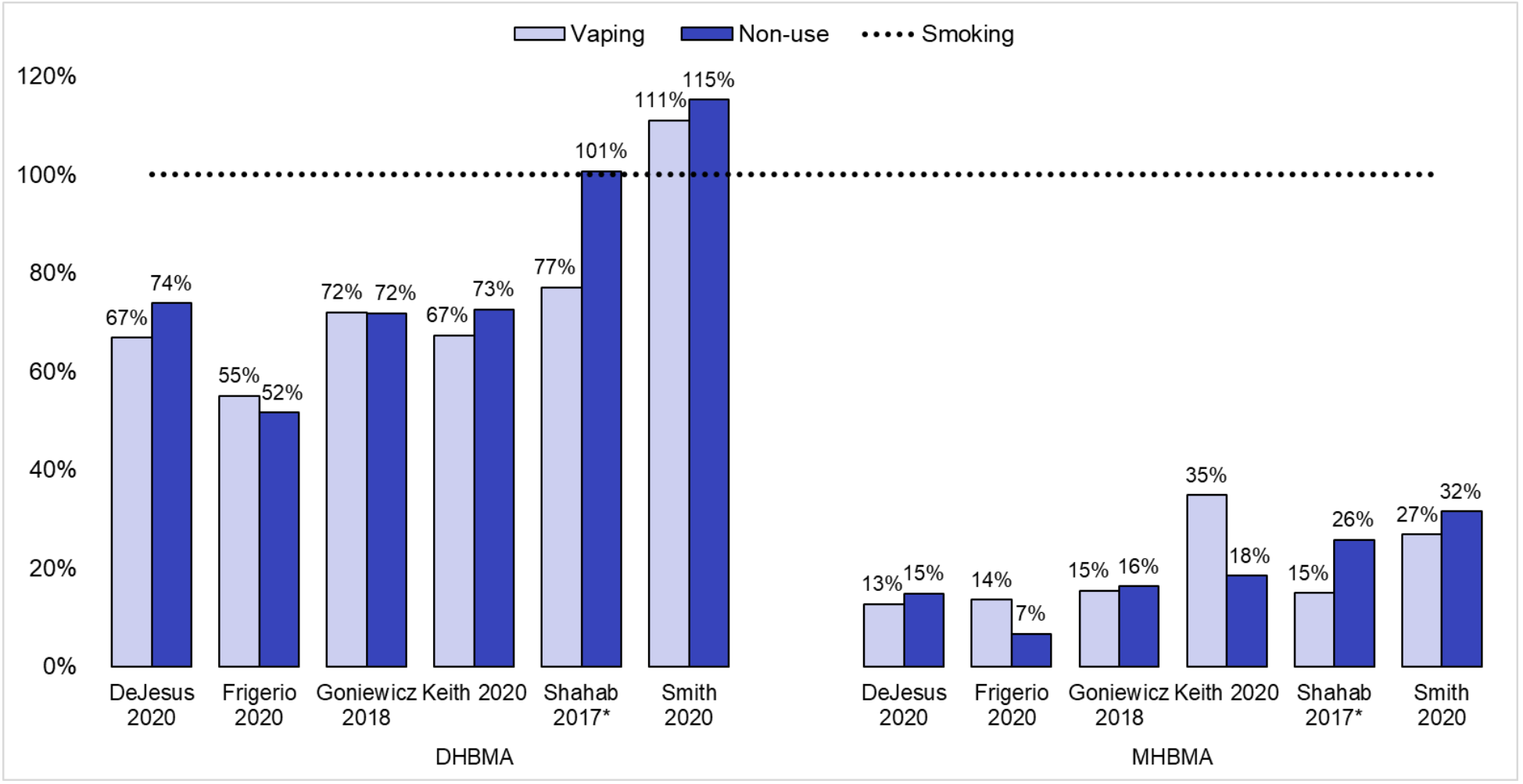
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 34. Levels of urinary 1,3-Butadiene biomarkers in vapers and non-users relative to smokers



Note: * Non-users in Shahab et al. (51) were using NRT.

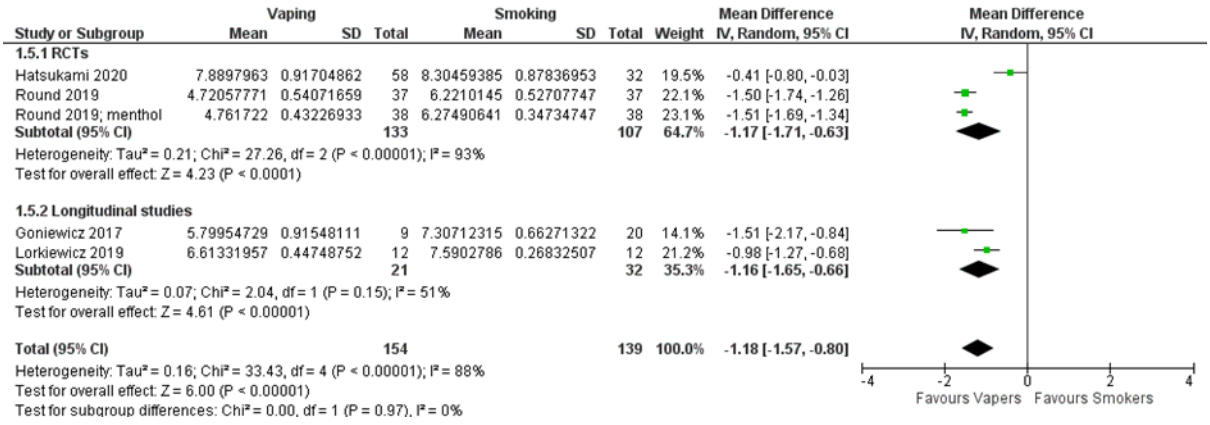
Crotonaldehyde (HMPMA, CMEMA)

Crotonaldehyde is possibly carcinogenic to humans according to the IARC (79) and is an acknowledged carcinogen according to the FDA (80). Tobacco smoke is the major source of exposure to crotonaldehyde, but it is also found in combustion products of vehicle fuel, wood, cooking fires, air pollution, some foods, heated cooking oils and in vaping product vapour (83). Exposure to crotonaldehyde potentially induces oxidative stress in human endothelial and bronchial epithelial cells and chronic inflammation in animal respiratory epithelium (83). Main metabolites of crotonaldehyde include 3-hydroxy-1-methylpropylmercapturic acid (HMPMA) and 2-carboxy1-1-methylethylmercapturic acid (CMEMA).

RCTs

Three RCTs reported on urinary HMPMA levels in smokers after they switched to vaping product use for 5 (7) and 7 days (8) in confinement and for 8 weeks ad libitum use (5) (table 19). We meta-analysed data from 2 RCTs comparing vaping and smoking groups' exposure to crotonaldehyde measured by urinary HMPMA levels (figure 35).

Figure 35. Meta-analysis of RCTs and longitudinal studies reporting on urinary HMPMA levels (crotonaldehyde) after exposure to vaping and smoking



The average urinary HMPMA levels were statistically significantly lower among vapers than smokers in 3 comparisons from 2 RCTs, where one RCT reported exposure levels among vapers who used tobacco- or menthol-flavoured e-liquids (7) (LMD: -1.17, 95% CI: -1.71, -0.63; 240 participants); the geometric mean HMPMA levels were approximately 69% lower among vapers than among smokers (GMR: 0.31, 95% CI: 0.18, 0.53). Heterogeneity was high at I^2 = 93%, but the direction of the difference was consistent across both studies. Also, findings from an RCT conducted in confinement with 5-day follow-up period suggested larger reduction in HMPMA levels in vapers compared with smokers (7) than results from the RCT that explored ad libitum use of vaping product for 8 weeks (5). An RCT in confinement by McEwan and others also reported a statistically significant reduction by around 88% in HMPMA levels 7 days after switching from smoking

to vaping product use; a similar reduction was observed among smokers who switched to HTP use and nicotine abstinence (8).

Cross-over studies

A cross-over study by St. Helen and others (75) reported 2.77 times (177%) higher HMPMA levels after smoking a cigarette than after drawing 15 puffs from a cartridge or pod vaping product or 10 puffs from a tank vaping product (cigarette to vaping product GMR: 2.77; 95% CI: 2.34, 3.29; table 19). HMPMA levels did not differ between single use of a vaping product and abstinence conditions (vaping product to abstinence GMR: 1.08, 95% CI: 0.94, 1.25).

Longitudinal studies

Three longitudinal studies reported on changes in urinary HMPMA levels after a single vaping product use (77), and 2 (23) and 4 weeks (21) after completely switching from smoking to using a vaping product (table 19). The meta-analysed data from 2 studies showed that the average urinary HMPMA levels were statistically significantly lower among vapers than smokers (LMD: -1.16, 95% CI: -1.65, -0.66; 53 participants; figure 35); the geometric mean HMPMA levels were approximately 69% lower among vapers than among smokers (GMR: 0.31; 95% CI: 0.19, 0.52). Heterogeneity between the 2 studies was substantial at I^2 = 51%, but the direction of the difference was consistent between the 2 studies. Although Pulvers and others reported a reduction in HMPMA levels withing vapers' group at 4-week follow-up, they reported no statistically significant differences in HMPMA changes between vapers and dual users' groups (21), which might be due to relatively small sample sizes at the last study follow-up (6 vapers and 21 dual users).

Table 19. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of crotonaldehyde among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
HMPMA							
Urine biosample							
RCT							
Hatsukami et al., 2020, US (5)	8 weeks (S-M)	See: Hatsukami et al., 2020	n=58 2669.9 (2108.6-3380.6)[b] pmol/mg creatinine (U) ↓48.4%	n=65 3545.2 (2924.9-4297.1)[a] ↓16.2%	n=32 4042 (2981.7-5480.4) ↑17.4%	NA	n=53 3316.1 (2551.3-4310.2) ↓37.7%
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 0.06[c] mg/24h (U) ↓**88.7%**	NA	n=30 0.54[a,d,e] 0.0%	n=29 0.05[c] ↓**91.5%**	n=28 0.07[c] ↓**87.0%**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 129.9	NA	NA	NA	Non-menthol, n=38: 118.9

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			(75.7) µg/24h (U) ↓**77.5%** Menthol, n=38: 128.4 (56.5) µg/24h (U) ↓**77.2%**				(37.6) ↓**77.9%** Menthol, n=40: 128.9 (56.5) ↓**76.5%**
Cross-over							
Helen et al., 2020, US (75)	Single use (A)	See: Helen et al., 2020	n=36 168.1 (95.4)[c] ng/mg creatinine (U) ↑15.5%%	NA	n=36 489.9 (297.7)[a] ↑236.5%	n=36 145.6 (55.3)	NA
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 Mean=502 µg/g creatinine (U)	n=11 709 ↓**64.7%**	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
			↓70.0%				
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 823.4 (387.7)[c] ng/mg creatinine (U)	NA	n=12 2051.4 (560.5)[a,d]	n=11 688 (161.6)[c]	n=12 923 (453)
Pulvers et al., 2018, US (21)	4 weeks (S-M)	See: Pulvers et al., 2018	n=6 Median (IQR) = 160.82 (154.5; 169.7) ng/mg creatinine (U) ↓47.0%	n=21 305.7 (228.6; 918.7) ↑0.8%	NA	NA	n=10 252 (157.8; 765.9) ↓16.9%

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

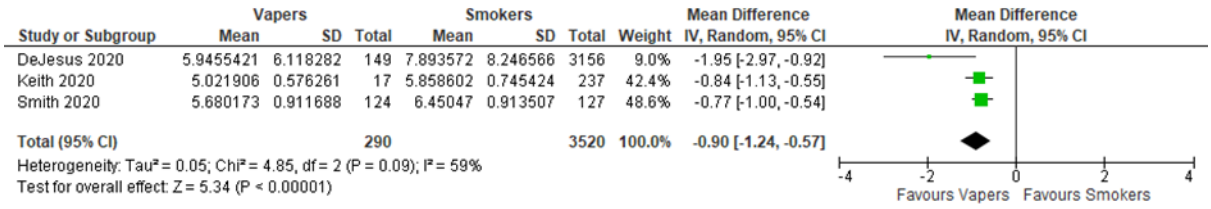
Cross-sectional studies

Seven studies reported on levels of crotonaldehyde, all of them reported on levels of the metabolite HMPMA (34-36, 50-52, 78), and one reported on levels of CMEMA (35) (table 20).

Six studies reported on levels of HMPMA among vapers and smokers (34-36, 51, 52, 78). Five studies reported statistically significantly different HMPMA levels between vapers and smokers, with levels reported to be between approximately 54% (52) and 86% lower (35) among vapers compared to smokers. De Jesus and others reported around 86% lower levels among vapers compared to smokers, however this was not tested for statistical significance (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies were pooled to assess urinary HMPMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary HMPMA level was 59% lower among vapers compared to smokers (LMD= -0.90, 95% CI -1.24, -0.57, p<0.001; figure 36). There was moderate heterogeneity between studies (I^2= 59%).

Figure 36. Meta-analysis of cross-sectional studies reporting on urinary HMPMA levels between vapers and smokers

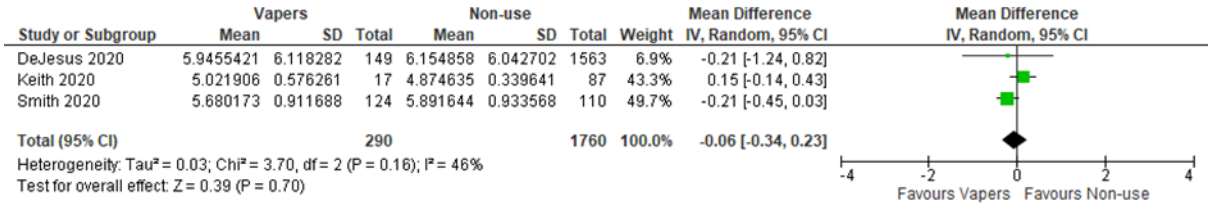


Frigerio and others reported that levels of CMEMA were statistically significantly lower, by 42%, among vapers compared to smokers (35).

Seven studies reported levels of HMPMA among vapers and non-users (34-36, 50-52, 78). Rubinstein and others reported levels to be statistically significantly higher by approximately 48% among adolescent vapers compared to non-users (50). Among studies reporting on levels among adults, urinary HMPMA levels were reported to be between approximately 3% (36) and 55% lower (51) among vapers compared to non-users, however none of these comparisons were statistically significant.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies were pooled to assess urinary HMPMA (34, 52, 78). Combining the 3 studies, the pooled geometric mean urinary HMPMA level was 6% lower among vapers compared to non-users (LMD= -0.06, 95%CI -0.34, 0.23, p=0.70; figure 37). The differences were not statistically significant. There was moderate heterogeneity between studies (I^2= 46%).

Figure 37. Meta-analysis of cross-sectional studies reporting on urinary HMPMA levels between vapers and non-users



Frigerio and others reported that urinary CMEMA levels were 15% non-significantly lower among vapers compared to non-users (35).

Across cross-sectional studies that measured urinary crotonaldehyde biomarker HMPMA, vapers' levels were approximately between 14% and 46% and non-users' levels were approximately between 18% and 57% of HMPMA levels detected among smokers. One study (35) reported that vapers' levels of CMEMA were approximately 58% and non-users' levels were 68% of those reported among smokers (figure 38).

Table 20. Cross-sectional studies reporting on levels of biomarkers of crotonaldehyde among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
HMPMA					
Urine biosample					
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	382 (37.2) ng/mL (U)	NA	2680 (67.9) 0.143	471 (19.4) 0.811
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th IQR): 38 (19; 133)[c] µg/g creatinine (U)**	NA	**268 (96; 580)[a,d]** **0.142**	**48 (15; 265)[c]** **0.792**
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**442.8 (387.6-505.8)[c] ng/mg creatinine (U)**	**2707.7 (2515.8-2914.3)[c]** **0.164**	**2359.3 (2188.2-2543.8)[a,b]** **0.188**	**457.7 (433.4-483.3)** **0.967**
Keith et al., 2020, US (34)	See: Keith et al., 2020	**179.1 (112.4)[b,c] ng/mg creatinine (U)**	**433.5 (399.8)[a,d]** **0.413**	**462.4 (398.6)[a,d]** **0.387**	**138.7 (49.5)[b,c]** **1.291**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
Rubinstein et al., 2018, US (50)	See: Rubinstein et al., 2018	**Median (IQR): 148.7 (99)[d] ng/mg creatinine (U)**	**185.4 (156.6)** **0.802**	NA	**100.4 (129.9)[a]** **1.481**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**235.9 (179.1-310.7)[b,c] ng/mg creatinine (U)**	**1200 (881.9-1631.6)[a,d]** **0.197**	**804.2 (563.8-1147.1)[a,d]** **0.293**	**366 (266-504.5)[b,c]** **0.645**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**293 (249–344)[b,c] ng/mg creatinine (U)**	**665 (554–799)[a,d]** **0.441**	**633 (540–742)[a,d]** **0.463**	**362 (305–431)[b,c]** **0.809**
CMEMA					
Urine biosample					
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th IQR): 233 (154; 542) µg/g creatinine (U)**	NA	**400 (220; 774)[d]** **0.583**	**273 (122; 603)[c]** **0.853**

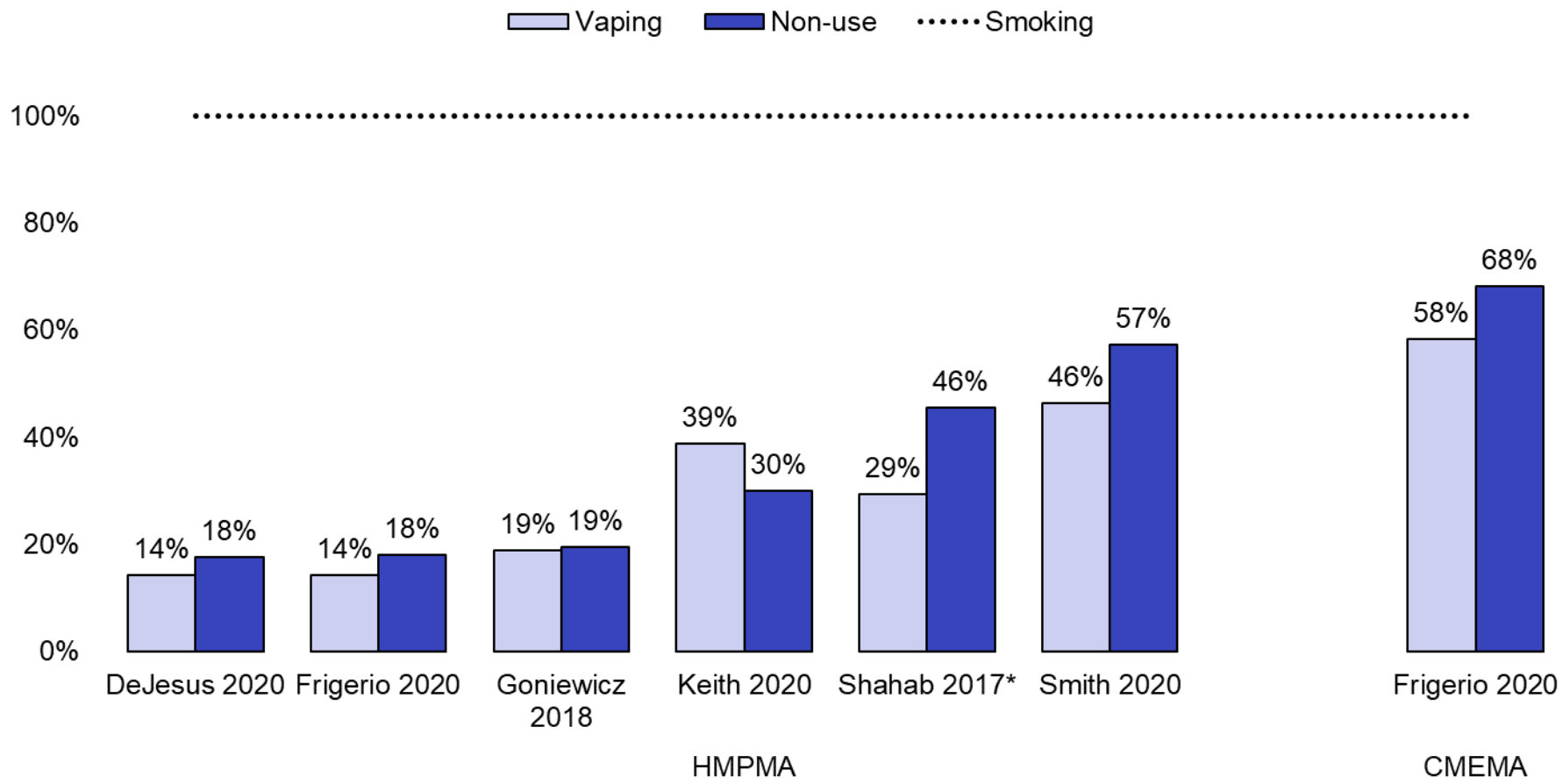
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 38. Levels of urinary crotonaldehyde biomarkers in vapers and non-users relative to smokers



Note: * Non-users in Shahab et al. (51) were all using NRT.

Formaldehyde (Formate)

Formaldehyde is carcinogenic to humans according to the IARC (79) and is a carcinogen according to the FDA (80). Formate is a metabolite used to reflect exposure to formaldehyde. Tobacco smoke contains high levels of formaldehyde which is produced when additives in tobacco, such as sugars, are burnt. Formaldehyde is also present in everyday household objects, such as glues, paints and cleaning fluids, therefore low levels are present in indoor and outdoor air and might confound the exposure from tobacco products. We reviewed several studies relating to formaldehyde in our 2018 report (1) where we explained how formaldehyde can be produced by dry puffing of vaping products.

Longitudinal studies

Two longitudinal studies assessed changes in urinary formate levels after single vaping product use (77) and vaping product use for a week (24) (table 21). Lorkiewicz and others did not detect changes in urinary formate levels after single use of a vaping product or smoking, while Dawkins and others reported higher urinary formate levels after using a 6mg/mL nicotine e-liquid with an adjustable-power vaping product for a week than after using an 18mg/mL nicotine e-liquid with the same type of vaping product for a week. Authors of the latter study concluded that formaldehyde exposure might increase during compensatory puffing behaviour with lower nicotine strength e-liquids (24).

Table 21. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of formaldehyde among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
Formate							
Urine biosample							
Longitudinal							
Dawkins et al., 2018, UK (24)	1 week (A)	See: Dawkins et al., 2018	1) 6mg/mL, fixed power, n=20 10.5 (8) µg/mg creatinine (U) 2) 18mg/mL, fixed power, n=20 9.6 (7.3) 3) 6mg/mL, adjustable power, n=20 18.0 (23.6) 4) 18mg/mL, adjustable power, n=20 7.6 (7.2)	NA	NA	NA	NA
Lorkiewicz et al.,	Single use	See: Lorkiewicz et al., 2019	n=12	NA	n=12	n=11	n=12

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
2019, US (77)	(A)		27.3 (24.3) ng/mg creatinine (U)		37 (32)	29.7 (27.5)	32.2 (15.7)

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Isoprene (IPM3)

Isoprene is possibly carcinogenic to humans according to the IARC (79) and is a carcinogen according to the FDA (80). N-acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-l-cysteine (IPM3) is a urinary metabolite of isoprene.

Cross-sectional studies

Two studies reported on the urinary isoprene metabolite IPM3 (36, 78) (table 22).

Both studies reported on IPM3 levels between vapers and smokers (36, 78). Goniewicz and others reported levels to be statistically significantly lower by approximately 88% among vapers compared to smokers (36) and De Jesus and others reported levels to be 92% lower among vapers compared to smokers, however this was not tested for statistical significance (78).

Both studies also reported on IPM3 levels among vapers and non-users (36, 78). Goniewicz and others reported levels to be 11% higher among vapers compared to non-users, which was not statistically significant (36). De Jesus and others reported levels to be 8% lower among vapers, however this was not tested for statistical significance.

Across cross-sectional studies that measured urinary isoprene biomarker IPM3, vapers' levels were approximately between 7.6% and 11.2% and non-users' levels were approximately between 8.3% and 10.1% of IPM3 levels detected among smokers (figure 39).

Table 22. Cross-sectional studies reporting on levels of biomarkers of isoprene among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
IPM3					
Urine biosample					
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	3.15 (0.31) ng/mL (U)	NA	41.2 (1.23) 0.076	3.43 (0.137) 0.918
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**3.747 (3.247-4.323)[c] ng/mg creatinine (U)**	**39.79 (36.33-43.56)[c]** **0.094**	**33.50 (30.69-36.56)[a,b]** **0.112**	**3.378 (3.155-3.617)** **1.109**

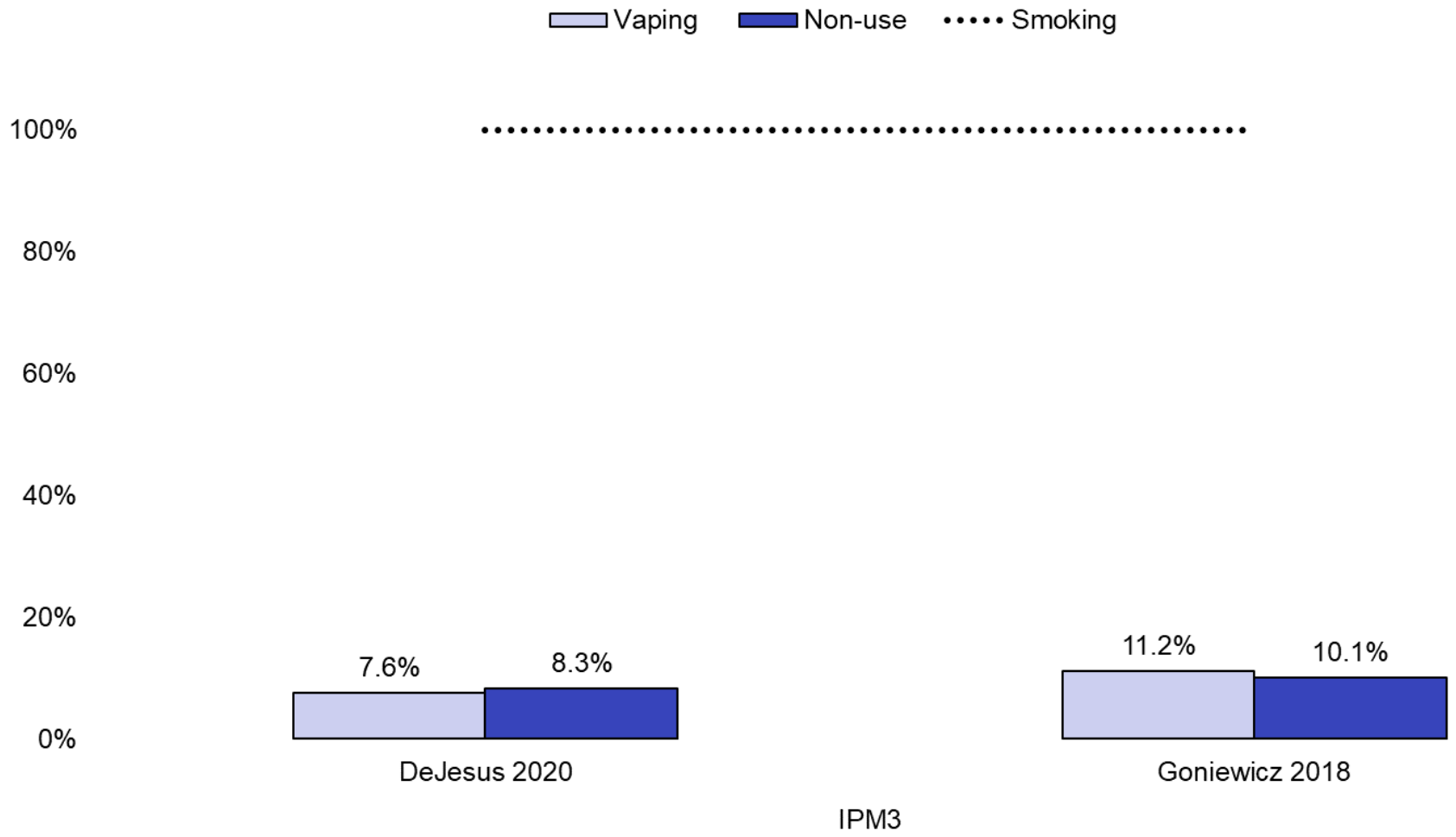
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 39. Levels of urinary isoprene biomarker IPM3 in vapers and non-users relative to smokers



Toluene (S-BMA)

Toluene is a respiratory, reproductive or developmental toxicant according to the FDA (80). The main metabolite of toluene is S-benzylmercapturic acid (S-BMA).

Longitudinal studies

A single longitudinal study reported on S-BMA levels after single use of a vaping product, smoking a tobacco cigarette and using a tobacco pouch (77) (table 23). The reported average S-BMA levels did not differ between non-users and after a single use of a vaping product, tobacco cigarette or tobacco pouch.

Table 23. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of toluene among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			Mean (SD or 95% CI)				
S-BMA (Toluene)							
Urine biosample							
Longitudinal							
Lorkiewicz et al., 2019, US (77)	Single use (A)	See: Lorkiewicz et al., 2019	n=12 41303 (26037) ng/mg creatinine (U)	NA	n=12 46104 (36272)	n=11 42401 (21694)	n=12 50445 (29788)

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

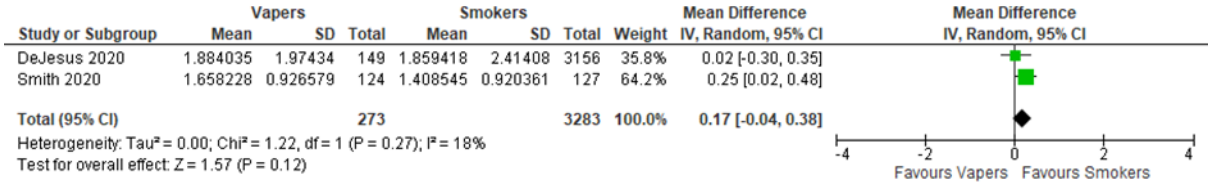
Cross-sectional studies

Four studies reported on the toluene metabolite S-BMA (35, 36, 52, 78) (table 24).

All 4 studies reported levels of S-BMA among vapers and smokers (35, 36, 52, 78). Levels were reported to be on average between 4% lower (35) and 28% higher (52) among vapers compared to smokers, however neither comparison was statistically significant. De Jesus and others reported levels were 3% higher among vapers compared to smokers, however this was not tested for statistical significance (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 2 studies were pooled to assess urinary S-BMA (52, 78). Combining the 2 studies, the pooled geometric mean urinary S-BMA level was 19% higher among vapers compared to smokers (LMD=0.17, 95% CI -0.04, 0.34, p=0.12; figure 40). The difference was not statistically significant. There was little heterogeneity between studies (I^2= 18%).

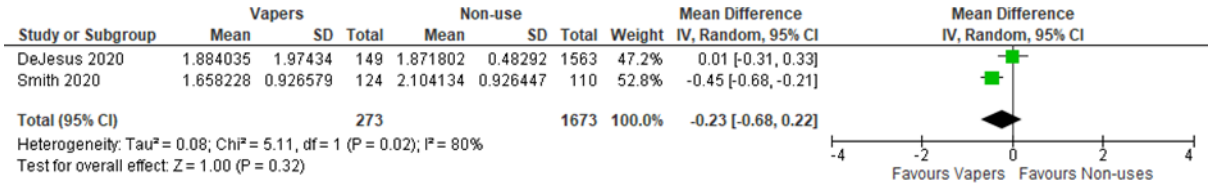
Figure 40. Meta-analysis of cross-sectional studies reporting on urinary S-BMA levels between vapers and smokers



All 4 studies reported levels of S-BMA among vapers and non-user (35, 36, 52, 78). Frigerio and others reported levels to be around 36% lower among vapers compared to non-users (35), and Smith and others reported levels were statistically significantly lower, by around 36%, among vapers compared to non-users (52). Goniewicz and others reported that S-BMA levels among vapers were statistically significantly higher, by on average 11%, among vapers compared to non-users (36). De Jesus and others reported no difference in S-BMA levels between vapers and non-users (78).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 2 studies were pooled to assess urinary S-BMA (52, 78). Combining the 2 studies, the pooled geometric mean urinary S-BMA level was 21% lower among vapers compared to non-users (LMD= -0.23, 95%CI -0.68, 0.22; p=0.32; figure 41). The difference was not statistically significant. There was substantial heterogeneity between studies (I^2= 80%). As discussed, there was variation in the definitions of non-use between studies.

Figure 41. Meta-analysis of cross-sectional studies reporting on urinary S-BMA levels between vapers and non-users



Across cross-sectional studies that measured urinary toluene biomarker S-BMA, vapers' levels were approximately between 97% and 128% and non-users' levels were approximately between 101% and 200% of S-BMA levels detected among smokers (figure 42).

Table 24. Cross-sectional studies reporting on levels of biomarkers of toluene among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
		Mean (SD or 95% CI)			
S-BMA					
Urine biosample					
De Jesus et al., 2020, US (78)	See: De Jesus et al., 2020	6.58 (0.59) ng/mL (U)	NA	6.42 (0.199) 1.025	6.5 (0.242) 1.012
Frigerio et al., 2020, Italy (35)	See: Frigerio et al., 2020	**Median (5th; 95th IQR): 1.42 (0.4; 4.28) µg/g creatinine (U)**	NA	**1.47 (0.53; 2.96)** **0.966**	**2.22 (0.55; 12.74)** **0.640**
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**6.985 (6.088-8.015)[d] ng/mg creatinine (U)**	**7.394 (6.836-7.998)[c]** **0.945**	**6.238 (6.238-7.188)[b]** **1.120**	**6.314 (5.965-6.683)[a]** **1.106**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**5.25 (4.45–6.18)[d] ng/mg creatinine (U)**	**4.72 (3.92–5.68)[d]** **1.112**	**4.09 (3.48–4.80)[d]** **1.284**	**8.20 (6.89–9.75)[a,b,c]** **0.640**

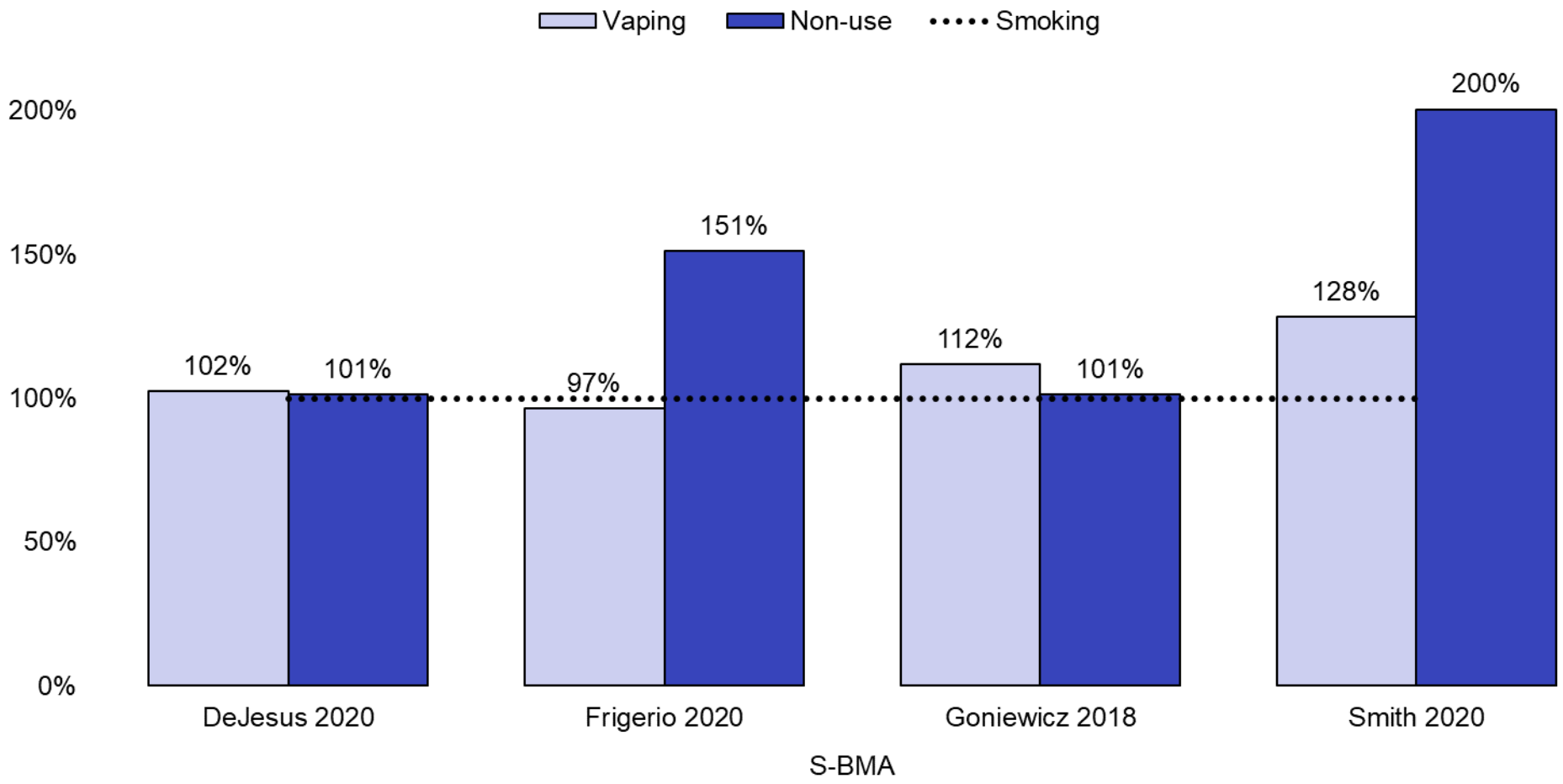
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 42. Levels of urinary toluene biomarker S-BMA in vapers and non-users relative to smokers



Summary of studies reporting on exposure to volatile organic compounds

In total 10 interventional or longitudinal observational studies reported on vaping product use associations with exposure to volatile organic compounds, 4 of which were funded by the tobacco industry. The included data suggest that switching from smoking to vaping product use substantially reduces exposure to volatile organic compounds. Acute vaping product use exposes users to substantially lower levels of all tested VOCs compared with smoking. After switching from smoking to exclusive vaping product use for up to a week in confined settings, the magnitude of the reduction in VOCs exposure ranged from 35% for acrylamide to over 80% for acrylonitrile, benzene and 1,3-butadiene. The reductions in exposure to nearly all tested VOCs were significant but smaller in magnitude in studies that explored switching from smoking to ad libitum vaping product use for follow-up periods longer than a week. Limited evidence on exposure to formaldehyde and toluene suggested no difference between vaping product use, smoking and non-use.

There was limited evidence from a single cross-over trial on exposure to VOCs differences after single vaping product use sessions compared with no use of tobacco or nicotine products. Compared with non-users, a single vaping product use session exposed users to significantly higher levels of acrylamide and benzene, to similar levels of acrolein and crotonaldehyde, and to lower levels of acrylonitrile and 1,3-butadiene.

There was substantial variation in the levels of VOCs reported by the 13 cross-sectional studies (one of which was tobacco industry funded). However, when studies were pooled, levels of acrylamide, acrolein, 1,3-butadiene and crotonaldehyde were all statistically significantly lower among vapers compared to smokers by on average at least one third. Acrylonitrile was substantially lower among vapers (by approximately 94%) compared to smokers, but over 3 times higher among vapers compared to non-users. Metabolites of benzene did not differ statistically significantly among vapers and smokers or vapers and non-users. There was little difference in the levels of all other VOC metabolites between vapers and non-users.

Levels among adolescents from one study (50), were broadly in the same direction to levels reported among adults. However, differences between vapers and non-users were greater among adolescents for acrylamide, acrolein and crotonaldehyde than adults. This may be due to the differences in vaping and smoking patterns among adolescents, but also differences in sample sizes and methodology, such as the use of NNAL to bio-verify non-use among adolescents, which was not utilised by any of the adult studies.

For the one study that examined vapers with respiratory symptoms, there was little difference in CEMA levels compared with non-users with respiratory symptoms whereas levels of CNEMA were higher among vapers with respiratory symptoms compared with

non-users with respiratory symptoms, but no statistical testing was carried out for these comparisons.

7.5 Biomarkers of exposure to tobacco-specific nitrosamines

TSNAs are formed from tobacco alkaloids during tobacco plant development, mainly through the nitrosation of nicotine during processing and curing of the tobacco plant. TSNAs are present in combusted and smokeless tobacco products. They are released in the air when tobacco is burned, so non-smokers are exposed through second-hand smoke (89). TSNAs have been found in trace amounts in aerosols from vaping products and oral nicotine replacement therapy products (89).

Study characteristics

The literature search identified 6 RCTs (4-8, 74), 2 cross-over trials (11, 15), 4 non-randomised longitudinal studies (18, 20, 21, 23) and 16 cross-sectional studies (29, 31-33, 36, 41, 44, 46, 47, 50-52, 54, 55, 62, 90) reporting on levels of biomarkers of tobacco-specific nitrosamines.

Of the 12 RCTs, cross-over and non-randomised longitudinal studies reporting on biomarkers of tobacco-specific nitrosamines, 8 were conducted in the US (4-7, 15, 20, 21, 74), 2 in the UK (8, 18), one in Canada (11) and one in Poland (23). Three RCTs (7, 8, 74) and one non-randomised longitudinal study (18) were funded by the tobacco industry (appendices: table 5).

One cross-sectional study was conducted in the UK (51) and one was from South Korea (62). A study by Smith and others (52) pooled data from Poland and the US and included participants from the UK, previously reported by Shahab and others (51). Thirteen studies reported data from the US (29, 31-33, 36, 41, 44, 46, 47, 50, 54, 55, 90). Of the 13 studies from the US, 5 reported on data from Wave 1 of the PATH study (36, 47, 54, 55, 90). One study was funded by the tobacco industry (44).

Sample sizes of the included studies ranged from 20 in a longitudinal study (23) to 520 in an RCT (4), participants' age ranged from a mean of 30.1 years (21) to a median of 47 years (5), and between 27% (21) and 60% of participants were women (23). All studies explored biomarkers of tobacco-specific nitrosamines in participants from non-specific general populations except for an RCT by Pulvers and others, which explored exposure among African American and Latinx smokers (6).

Across cross-sectional studies, sample sizes ranged from 44 (41) to 11,104 (90). Adult participants' mean age ranged from 29.8 years (41) to 40.4 years (46), and participants in 2 studies that included adolescents, reported mean ages of 16.4 years (50) and 15.8 years (32). Between 24% (62) and 100% (47) of participants from the general population studies

were women. Seven studies reported on specific populations or specific comparisons. One focused on differences between pregnant and non-pregnant women (54), and another on pregnant women (33), one investigated differences between those with and without respiratory symptoms (55), 2 reported on adolescents (32, 50), one sampled participants of ‘American Indian’ descent (31) and one compared differences between types of vaping products (44).

RCTs

A total of 1366 participants were recruited across the 6 RCTs (4-8, 74). All RCTs recruited participants who smoked at least 5 (5, 6) or 10 cigarettes per day (4, 7, 8, 74). All 3 tobacco-industry-funded RCTs were conducted in confinement with 5 (7, 74) or 7 days follow-up (8), while independently funded studies followed up participants for 6 (6), 8 (5) or 24 weeks (4). The RCTs randomised participants to vaping, dual use, smoking, use of NRT, use of HTP or abstinence.

Cross-over studies

Two cross-over studies (11, 15) reported on exposure to tobacco-specific nitrosamines among a total of 70 participants. Both studies recruited dual users who on average smoked at least 5 (11) or 10 cigarettes (15) per day, and included 5 (15) and 7 day (11) long cross-over conditions of vaping, smoking, dual use and non-use of tobacco or nicotine products.

Longitudinal studies

The 4 non-randomised longitudinal studies in total recruited 319 participants (18, 20, 21, 23). Three studies included smokers of at least 5 cigarettes per day (18, 21, 23), while Jacob and others reported on 2 separate studies that included vapers and dual users (20). Studies included different follow-up lengths—acute exposure and 3 to 5 days follow-up (20), 2 weeks (23), 4 weeks (21) and 24 months (18). Levels of biomarkers of tobacco-specific nitrosamines were reported for vapers and dual users, including participants who relapsed after initially switching to vaping.

Cross-sectional studies

Across the 15 studies that measured tobacco-specific nitrosamine levels among vapers, there was wide variation in the measurements of participants frequency of vaping and smoking. Four studies compared levels among daily users (29, 31, 52, 90), 5 among people who used vaping products everyday or on some days (36, 47, 54, 55, 62), 2 among people who used vaping products at least weekly (32, 51), 2 among people who used vaping products at least monthly (33, 50) and 2 did not define the frequency of vaping product use of participants (41, 44).

Across the 11 studies that included non-using participants, 3 required participants to have never smoked or vaped (41, 47, 90), and 4 did not define participants past tobacco or vaping product use requirements (36, 50, 52, 55). One defined non-users as those who had not smoked for at least 7 days (32), one where participants had not smoked for 6 months (29), and one where participants had not smoked for 6 months and were using NRT (51). One included those who were ex-smokers and those who had never smoked (62).

Risk of bias in included studies

RCTs

All 6 RCTs were assessed to have some concerns in relation to overall risk of bias according to the RoB2 risk of bias tool (appendices: table 1). Key concerns were a lack of information on the randomisation process and pre-specified data analysis plans.

Cross-over studies

Both cross-over studies that reported on metabolites of tobacco-specific nitrosamines (11, 15) were assessed to be at high risk of bias according to the RoB2 risk of bias tool for cross-over studies due to potential deviations from intended interventions (appendices: table 2). The key issue was that participants' adherence to the non-use condition was only confirmed by self-report.

Longitudinal studies

Of the 4 included non-randomised longitudinal studies, 3 were assessed at moderate (18, 21, 23) and one (20) at serious risk of bias according to the ROBINS-I risk of bias tool (appendices: table 3). The Jacob and others study was judged to be at serious risk of bias as it did not account for potential confounding due to participants' smoking.

Cross-sectional studies

The quality of all cross-sectional studies was assessed using Biocross quality appraisal tool and is reported in appendices (appendices: table 4). Studies reporting levels of TSNAs scored between 9 (50) and 16 (52) out of a maximum score of 20, with most studies of reasonably good quality. The main limitations were associated with lack detail about statistical adjustments for confounders and limited detail on laboratory measurement procedures (blinded analyses, reporting on quality control procedures).

Study findings

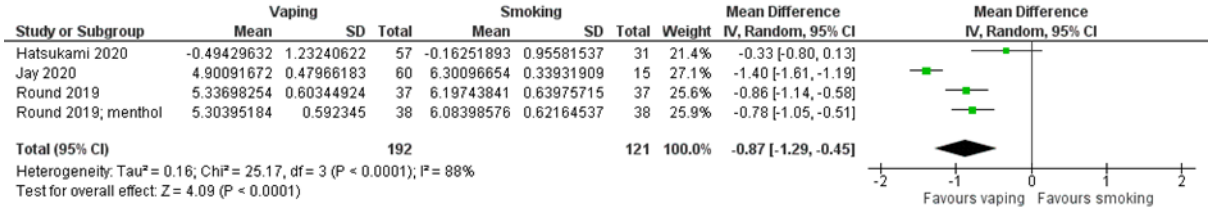
NNK (NNAL)

Both 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and its metabolite 4 (methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) are carcinogenic to humans according to the IARC (79) and are categorised as carcinogens by the FDA (80). NNK and NNAL are both powerful pulmonary carcinogens in animals and humans (72). NNAL is reported to have a half-life of approximately 10 days (36).

RCTs

All 6 included RCTs reported on urinary NNAL levels in vapers and other groups (table 25). We pooled data from 3 RCTs (one providing 2 comparisons) that matched our criteria for meta-analysis (methods: table 6) and compared urinary NNAL levels in vaping and smoking groups after 5 days in confinement (7, 74) and 8 weeks of ad libitum product use (5) (figure 43).

Figure 43. Meta-analysis of RCTs reporting on urinary NNAL levels (NNK) after exposure to vaping and smoking



The average urinary NNAL levels were statistically significantly lower among vapers than smokers (LMD: -0.87, 95% CI: -1.29, -0.45; 313 participants); the geometric mean NNAL levels were approximately 58% lower among vapers than among smokers (GMR: 0.42, 95% CI: 0.28, 0.64). Heterogeneity was high at I^2 = 88%, but the direction of the difference was consistent across the 3 trials. Furthermore, the reduction in NNAL levels in vapers' groups compared with smokers appeared to be higher in the 2 RCTs conducted in confinement with 5-day follow-ups (7, 74) than in smokers who switched to ad libitum vaping product use for 8 weeks (5).

The largest RCT (4) randomised smokers of at least 10 cigarettes per day who were not planning to stop smoking to ad libitum use of a cartridge vaping product with 0mg/mL, 8mg/mL or 36mg/mL nicotine e-liquid and to using a cigarette substitute without nicotine for 24 weeks. As a complete switch from smoking to vaping product use was not enforced and participants at the last follow-up reported smoking between 6 and 10 cigarettes per day, average reductions in tobacco specific nitrosamines that were reported in this trial are associated with concurrent smoking and vaping product use rather than vaping product use only. At 24 weeks after randomisation, only participants within the 36mg/mL nicotine

e-liquid vaping group demonstrated a statistically significant reduction of around 45% in urinary NNAL levels. Pulvers and others also reported a statistically significant reduction of around 67% in average urinary NNAL levels 6 weeks after switching from smoking to using a pod vaping product, however only 28.1% in this group had completely switched from smoking to vaping, 57.9% were dual users and 14% continued smoking only (6). The third RCT (conducted in confinement for 7 days) (8) reported an average reduction of around 65% in urinary NNAL levels among the vapers group, slightly higher than reduction in the HTP group (~41%) and similar to the reduction among the abstinence group (~67%).

Cross-over studies

Both cross-over studies concluded that urinary NNAL levels were lower during vaping product use only and non-use conditions compared with dual use or smoking conditions (table 25). Czoli and others reported similar reduction patterns in NNAL levels for vaping and non-use (~29% and ~35%, respectively), while Cobb and others indicated a lower reduction within vaping compared with non-use (~40% and ~76%, respectively); these discrepancies between the studies may be due to some participants continuing to smoke during the vaping condition. Also, NNAL has a half-life of around 10 days (36), and both cross-over studies as well as the RCTs that were conducted in confinement, followed up participants for up to 7 days, which might have affected the measured urinary NNAL levels.

Longitudinal studies

All 4 non-randomised longitudinal studies reported on NNAL changes in participants who switched to vaping (table 25), but due to lack of studies with similar design (see methods: table 6) their data could not be pooled for meta-analysis. In the inpatient study reported by Jacob and others, urinary NNAL levels after single use of a vaping product did not differ from baseline due to its half-life of over 10 days, and in the outpatient study the average reduction in NNAL levels after 3 to 5 days was not statistically significant, likely due to small sample size (n=11) and short (relative to NNAL half-life) follow-up (20). Two studies followed-up smokers who switched to using vaping product for 2 (23) and 4 weeks (21), and reported that reductions in urinary NNAL levels among vapers only were greater compared with studies that followed-up participants for shorter periods of time. Goniewicz and others reported a statistically significant reduction by approximately 78% in urinary NNAL levels at 2 weeks follow-up (23), and Pulvers and others reported an approximately 97% reduction in NNAL levels 4 weeks after switching from smoking to using vaping product (21). Both studies reported reduced urinary NNAL levels among dual users—participants who initially switched to vaping but later went back to concurrently smoking—but these changes were not statistically significant. Average levels of NNAL also dropped for the first few months after smokers switched to vaping product use in Walele and others (18), but then remained relatively stable until the last follow-up at 24 months.

Table 25. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of NNAL among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Urine biosample							
RCT							
Cobb et al., 2021, US (4)	24 weeks (S-M)	See: Cobb et al., 2021	a1) 8 mg/mL, n=73 250.17 (194.46-323.94) pg/mg creatinine (U) ↓29.7% a2) 36 mg/mL, n=79 196.37 (154.47-251.11)[d] ↓**45.3%** a3) 0 mg/mL, n=69 309.62 (237.43-406.7)	NA	NA	n=90 329.08 (260.68-417.73)[a2] ↓8.1%	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			↓14.1%				
Hatsukami et al., 2020, US (5)	8 weeks (S-M)	See: Hatsukami et al., 2020	n=57 0.61 (0.45-0.84) pmol/mg creatinine (U) ↓53.4%	n=65 0.81 (0.67-0.99) ↓28.9 %	n=31 0.85 (0.61-1.19) ↓17.5%	NA	n=53 0.67 (0.46-0.97) ↓55.0%
Jay et al., 2020, US (74)	5 days (A)	See: Jay et al., 2020	n=60 150.8 (76.7) ng/24h (U) ↓**67.5%**	NA	n=15 577.4 (201.7) ↑4.2%	n=11 159.5 (117.4) ↓**66.8%**	NA
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 82.29[c] ng/24h (U) ↓**65.1%**	NA	n=30 289.54[a,d,e] ↓9.8%	n=29 101.5[c,e] ↓**67.8%**	n=28 195.71[c,d] ↓**41.1%**
Pulvers et al., 2020, US (6)	6 weeks (S-M)	See: Pulvers et al., 2020	n=114 Median (IQR)=40 (12; 101)[c] pg/mL creatinine (U) ↓**67.7%**	NA	n=54 97 (39;222)[a] ↑10.2%	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 249.4 (165.3) ng/24h (U) ↓**58.6%** Menthol, n=38: 239.7 (155.4) ng/24h (U) ↓**55.0%**	NA	NA	NA	Non-menthol, n=38: 176.7 (113.1) ↓**63.6%** Menthol, n=40: 201.4 (115.8) ↓**60.0%**
Cross-over							
Cobb et al., 2020, US (15)	5 days (A)	See: Cobb et al., 2020	n=22 EEM (SEM)=94.2 (28.2) pg/mL (U) ↓39.6%	n=22 116.0 (27.2) ↑1.8	n=22 135.4 (27.2) ↓11.0%%	n=11 Mean (SEM)=23.78 (13.0) ↓75.7%%	NA
Czoli et al., 2019, Canada (11)	7 days (A)	See: Czoli et al., 2019	21.25[b,c] (14.34-31.47) pg/mg creatinine (U) ↓28.9%	Baseline: 30.26[a,d] (21.06-43.48)	32.76[a,d] (23.89-44.91) ↑8.3%	19.76[b,c] (13.45-29.03) ↓34.7%	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 Mean=45 ng/g creatinine (U) ↓**78.0%**	n=11 109 ↓55.0 %	NA	NA	NA
Jacob et al., 2020, US (20)	4 hours after single use & 3-5 days (A)	See: Jacob et al., 2020.	Inpatients, n=11 0.34 (0.83) pmol/mg creatinine (U) ↓22.7% Outpatients, n=40 0.24 (0.32) ↓11.1%	NA	NA	NA	NA
Pulvers et al., 2018, US (21)	4 weeks (S-M)	See: Pulvers et al., 2018	n=6 Median (IQR) = 3.5 (2; 20.3)[b,d] pg/mg creatinine (U) ↓96.6%	n=21 156.13 (52.5; 320.7)[a,d] ↑51.6 %	NA	NA	n=10 22.5 (4.7; 119.3)[a,b] ↓78.1%

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Walele et al., 2018, UK (18)	24 months (L)	See: Walele et al., 2018	n=102 Mean (SEM)=136 (15.6) ng/24h (U) ↓8.7%	NA	NA	NA	NA

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

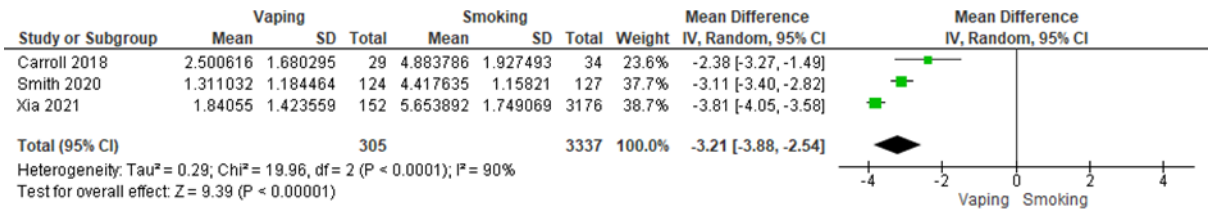
[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

Eleven studies compared levels of urinary NNAL levels between vapers and smokers (29, 31, 32, 36, 41, 47, 51, 54, 62, 64, 90) (table 26). Nine studies reported that urinary NNAL levels were statistically significantly lower among vapers compared to smokers, by approximately 74% (62) to 98% lower (36). Bustamante and others (29) reported levels to be around 94% lower among vapers compared to smokers, and Coleman and others (54) reported levels to be 93% lower among non-pregnant women who vape, and 92% lower among pregnant women who vape compared to those who smoke. Neither comparison was tested for statistical significance. Chaffee and others (32) reported levels to be 30% lower among adolescents who vaped in the past 7 days compared to adolescents who smoked in the past 7 days, differences were not tested for statistical significance. Oliveri and colleges reported levels of NNAL between vaping products, with marginally higher NNAL levels reported among those using cartridge models compared to those using tank models (44).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 3 studies measuring NNAL levels among daily vapers and smokers were pooled for meta-analysis (31, 52, 90). Combining the 3 studies, the pooled geometric mean urinary NNAL level was 96% lower among daily vapers compared to daily smokers (LMD= -3.21, 95% CI -3.88, -2.54; p<0.001; figure 44). There was substantial heterogeneity between studies (I^2= 90%). Although all 3 studies reported levels among daily vapers, Smith and others (52) used strict definitions of daily use—daily use for 6 months and use of more than 5 cartridges, one bottle of e-liquid or 2 disposable vaping products a week. Carroll and others (31) required participants to have vaped in the past 24 hours, whereas Xia and others (90) did not include such strict requirements. Moreover, Smith and others (52) and Carroll and others (31) both required carbon monoxide bio-verification for smoking and non-smoking status, whereas Xia and others (90) relied on self-report. Finally, there were methodological differences between sample collection techniques, with Carroll and others (31) and Smith and others (52) requiring participants to provide a sample during a laboratory visit, whereas participants in Xia and others study (90) collected samples at home and posted them to researchers.

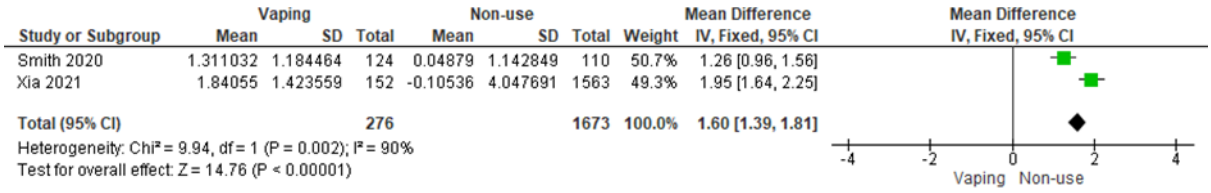
Figure 44. Meta-analysis of cross-sectional studies reporting on urinary NNAL levels between vapers and smokers



Twelve studies compared levels of NNAL between vapers and non-users (29, 32, 36, 41, 47, 50, 51, 54, 55, 62, 64, 90). Levels were reported to be statistically significantly higher among daily vapers than never smokers in a study by Xia and others (90). Eight studies reported levels to be around 1.69 times (62) to 42 times (41) significantly higher among vapers compared to non-users. Shahab and others (51) reported that NNAL levels were statistically significantly lower, by approximately 70%, among vapers compared to ex-smokers who were using NRT. Bustamante and others reported that levels of NNAL were 70 times higher among daily vapers compared to those who quit smoking at least 6 months prior, however this was based on a small sample (n=59), which was not adjusted for creatinine and was not tested for statistical significance (29). Dai and others found that vapers with self-reported respiratory symptoms had over 3.1-fold higher NNAL levels than among non-users with symptoms, but the study did not test for statistical significance; similarly, NNAL levels were around 3.4-fold higher among vapers without symptoms than among non-users without respiratory symptoms, but these differences were not tested for statistical significance (55). Rubinstein and others reported levels to be statistically significantly higher among adolescent vapers compared to non-users (50), and Chaffee and others reported levels of NNAL to be 1.91 times higher among adolescents who had vaped in the past 7 days compared to those who had not vaped or smoked (32).

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 2 studies were pooled to assess urinary NNAL between vapers and non-users (52, 90). Both studies compared levels among daily vapers. Across the 2 studies, the geometric mean urinary NNAL level was 395% higher among daily vapers than among non-users (LMD= 1.60, 95% CI 1.39, 1.81; p<0.001; figure 45). There was substantial heterogeneity between studies (I^2= 90%). Although both studies reported on daily vapers, Xia and others (90) compared to never smokers and Smith and others (52) compared to those who had not smoked for at least 6 months prior. As detailed above, there were also differences in definitions and methodology between the studies.

Figure. 45 Meta-analysis of cross-sectional studies reporting on urinary NNAL levels between vapers and non-users



One study reported levels of NNK in hair samples, reporting not statistically significantly difference in levels of NNK in samples from those who had vaped and smoked in the past month compared to those who had just smoked in the past month (33).

Across cross-sectional studies that measured urinary NNAL, vapers' levels were approximately between 2% and 70% and non-users' levels were approximately between 0% and 37% of NNAL levels detected among smokers (figure 51).

Table 26. Cross-sectional studies reporting on levels of NNK and its biomarker NNAL among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
NNAL					
Urine biosample					
Bustamante et al., 2018, US (29)	See: Bustamante et al., 2018	0.07 (0.18) pmol/mL (U)	NA	1.28 (1.04) 0.055	0.001 (0.001) 70.0
Carroll et al., 2018, US (31)	See: Carroll et al., 2018	**12.19 (6.62-22.47)[b,c] pg/mg (U)**	**147 (86.89-248.7)[a]** **0.083**	**132.13 (69.12-252.57)[a]** **0.092**	NA
Chaffee et al., 2019, US (32)	See: Chaffee et al., 2019	0.44 (0.16-1.20) pg/mg creatinine (U)	4.18 (1.38-12.62) 0.105	0.63 (0.28-1.39) 0.698	0.23 (0.21-0.26) 1.913

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
Coleman et al., 2021, US (54)	See: Coleman et al., 2021	Non-pregnant: 6.12 (3.59-10.41) pg/mg creatinine (U) Pregnant: 14.9 (2.04-108.67) pg/mg creatinine (U)	125.92 (96.61-164.17) 0.047 136.8 (80.43-232.7) 0.109	90.91 (71.58-115.45) 0.067 196.79 (130.32-297.1) 0.076	NA
Dai et al., 2020, US (55)	See: Dai et al., 2020	Without symptoms: 4.4 (3.5-5.6) pg/mg creatinine (U) With symptoms: 10.7 (6.5-17.5) pg/mg creatinine (U)	98.9 (79.7-122.7) 0.044 199.6 (176.7-225.4) 0.054	NA	1.4 (1.2-1.6) 3.143 3.1 (2.4-3.9) 3.452

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**4.887 (3.817-6.257)[c,d] pg/mg creatinine (U)**	**262.6 (240.0-287.3)[c]** **0.019**	**203.5 (181.7-227.9)[a,b]** **0.024**	**0.921 (0.819-1.035)[a]** **5.306**
Oliveri et al., 2020, US (44)	See: Oliveri et al., 2020	**Tank VP: 130.5 (194.7)[b] ng/g creatinine (U)** **Cartridge VP: 160.3 (245.7)[b] ng/g creatinine (U)**	**332.7 (331.6)[a]** **0.392 (vs tank VP)** **0.482 (vs cartridge VP)**	NA	NA
Perez et al., 2021, US (47)	See: Perez et al., 2021	**0.005 (0.004-0.007)[c,d] ng/mg creatinine (U)**	NA	**0.2 (0.1-0.2)[a,d]** **0.025**	**0.0009 (0.0008-0.001)[a,c]** **5.556**
Piper et al., 2019, US (46)	See: Piper et al., 2019	NA	**340.99 (387.86)[c] pg/mL (U)**	**453.31 (410.12)[b]** **1.33**	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
Reidel et al., 2017, US (41)	See: Reidel et al., 2017	**17.22 (6)**[c,d] **pg/mL (U)**	NA	**269.5 (67.72)**[a,d] **0.064**	**0.41 (0.22)**[a,c] **42.0**
Rubinstein et al., 2018, US (50)	See: Rubinstein et al., 2018	**Median (IQR): 0.3 (7)**[b,d] **pg/mL creatinine (U)**	**68.11 (68.7)**[a] **0.004**	NA	0a
Rudasingwa et al., 2021, South Korea (62)	See: Rudasingwa et al., 2021	**Median (IQR): 8.3 (4.9; 25.4)**[c,d] **pg/mL (U)**	NA	**32 (4.9; 69.8)**[a,d] **0.259**	**4.9 (4.9; 4.9)**[a,c] **1.694**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**1.47 (1.02-2.02)**[b,c,d] **pg/mg creatinine (U)**	**44.5 (28.5-69.4)**[a,d] **0.033**	**53.4 (36.6-77.8)**[a,d] **0.028**	**4.83 (2.79-8.34)**[a,b,c] **0.304**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**3.71 (3.02–4.57)**[b,c,d] **pg/mg creatinine (U)**	**48.5 (38.4–61.2)**[a,c,d] **0.076**	**82.9 (67.7–101.4)**[a,b,d] **0.045**	**1.05 (0.84–1.30)**[a,b,c] **3.533**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
Xia et al., 2021, US (90)	n = 11104 % within age groups: 18-24—31.9%, 25-34—21.1%, 35-54—30.2%, >55—15.9%, 45.5% females, 16.6% Hispanic, 15.0% non-Hispanic Black, 59.5% non-Hispanic White, 8.0% of other ethnicity. Vapers (n=152): daily use of VP. Dual users (n=1983): daily use of TC, VP or smokeless tobacco and daily or intermittent use of at least one another category. Smokers (n=3176): daily use of TC, cigar, cigarillo, filtered cigar, pipe, and/or hookah. Non-users (n=1563): never use of tobacco and nicotine products.	**6.3 (4.7-7.9)**[b,c,d] **ng/g creatinine (U)**	**278.6 (254.9-302.2)**[a,d] **0.023**	**285.4 (267.9-303.3)**[a,d] **0.022**	**0.9 (0.8-1.1)**[a,b,c] **7.0**
Hair biosample					
Clemens et al., 2019, US (33)	See: Clemens et al., 2019	NA	**0.030 (0.002-0.395) pg/mg (H)**	**0.005 (0.001-0.025)** **6.0**	**0.004 (0.001-0.013)** **7.5**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
NNK					
Hair biosample					
Clemens et al., 2019, US (33)	See: Clemens et al., 2019	NA	**0.213 (0.006-7.672)[d] pg/mg (H)**	**0.131 (0.019-0.888)** **1.626**	**0.003 (0.001-0.011)[b]** **71.0**

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Anabasine (NAB) and anatabine (NAT)

Both N-Nitrosoanabasine (NAB) and N-Nitrosoanatabine (NAT) are classified as group 3 carcinogens according to the IARC, which means they are not carcinogenic to humans (79).

RCTs

One RCT funded by the tobacco industry (7) measured changes in urinary NAB and NAT levels after switching from smoking at least 10 cigarettes per day to ad libitum use of a cartridge vaping product with 48mg/mL nicotine e-liquid for 5 days in confinement (table 27). Urinary NAB and NAT levels were statistically significantly reduced at day 5 by over approximately 86% and 97% respectively. A similar reduction for NAB and NAT levels was reported for participants who for 5 days switched from smoking to nicotine gum use (7).

Table 27. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of anabasine (NAB) and anatabine (NAT) among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
NAB							
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 5.8 (2.7) ng/24h (U) ↓**89.4%** Menthol, n=38: 6.4 (2.2) ng/24h (U) ↓**86.5%**	NA	NA	NA	Non-menthol, n=38: 6.1 (2.6) ↓**88.2%** Menthol, n=40: 6.9 (5.1) ↓**85.1%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
NAT							
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 3.9 (7.9) ng/24h (U) ↓**98.7%** Menthol, n=38: 5.6 (7.9) ng/24h (U) ↓**97.9%**	NA	NA	NA	Non-menthol, n=38: 2.4 (1) ↓**99.2%** Menthol, n=40: 4.6 (9) ↓**98.4%**

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

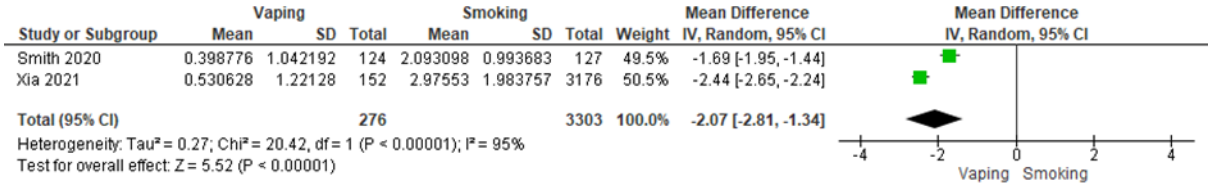
[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

Four cross-sectional studies compared urinary levels of NAB between vapers and smokers (36, 51, 64, 90) (table 28). Levels of NAB were lower among vapers compared to smokers in all 4 studies. Urinary NAB levels were statistically significantly lower, by approximately 82% (64) to 91% (36), among vapers compared to smokers. Xia and others (90) also reported levels to be 91% lower among daily vapers compared to daily smokers, however this was not tested for statistical significance.

Following the algorithm for selecting studies for meta-analysis (methods: table 4), 2 studies, both measuring levels among daily vapers and smokers, were pooled to assess urinary NAB (52, 90). Across the 2 studies, the pooled geometric mean urinary NAB level was 87% lower among daily vapers compared to daily smokers (LMD= -2.07, 95% CI -2.81, -1.34; p<0.001; figure 46). There was substantial heterogeneity between studies (I^2= 95%), however all estimates were in the same direction. Although both studies reported levels among daily vapers, Smith and others (52) used strict definitions of daily use, whereas Xia and others (90) did not have strict inclusion criteria.

Figure 46. Meta-analysis of cross-sectional studies reporting on urinary NAB levels between vapers and smokers

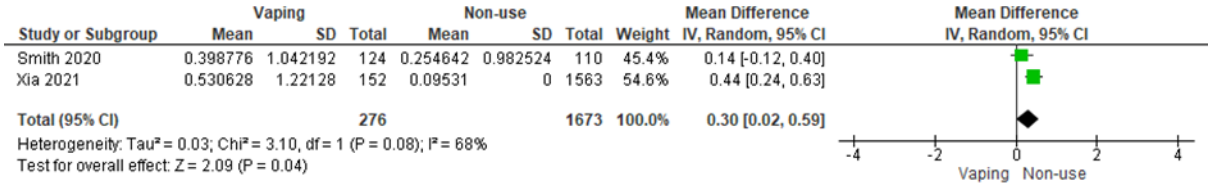


Four cross-sectional studies compared levels on NAB between vapers and non-users (36, 51, 64, 90) (table 28). Goniewicz and others reported levels to be statistically significantly higher among vapers compared to non-users, by around 33% (36). Smith and others reported levels to be approximately 16% higher among vapers, however this was not tested for statistical significance (64). Xia and others reported levels to be 54% higher among daily vapers compared to never smokers, however this was not tested for statistical significance (90). Shahab and others reported levels to be on average 30% lower among vapers when compared to ex-smokers who use NRT (51).

Following the algorithm for selecting studies for meta-analysis, 2 studies, both measuring levels among daily vapers and non-users, were pooled to assess urinary NAB (52, 90). Across the 2 studies, the pooled geometric mean urinary NAB level was 34% higher among daily vapers than among non-user (LMD= 0.30, 95% CI 0.02, 0.59; p=0.040; figure 47). There was substantial heterogeneity between studies (I^2= 68%). Although both studies reported on daily vapers, Xia and others (90) only included never smokers and Smith and others (52) included participants who have ceased smoking for at least 6

months. Moreover, as detailed above, there were distinct differences in methodology between the 2 studies.

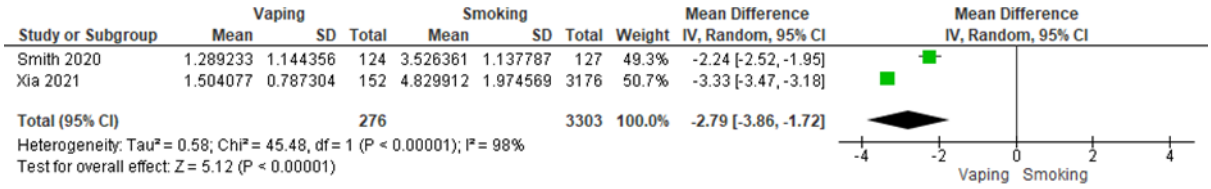
Figure 47. Meta-analysis of cross-sectional studies reporting on urinary NAB levels between vapers and non-users



Four cross-sectional studies compared levels on NAT between vapers and smokers (36, 51, 64, 90). Across 3 studies, NAT levels were statistically significantly lower among vapers compared to smokers, by approximately 90% (52) to 96% (36). Xia and others reported levels to be around 96% lower among daily vapers compared to daily smokers, however this was not tested for significance (90)

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 2 studies, both measuring levels among daily vapers and smokers, were pooled to assess urinary NAT (52, 90). Across the 2 studies, the pooled geometric mean urinary NAT level was 94% lower among daily vapers compared to daily smokers (LMD= -2.79, 95% CI -3.86, -1.72; p<0.001; figure 48). There was substantial heterogeneity between studies (I^2= 98%). Although both studies reported levels among daily vapers, as reported above, there were methodological differences between the studies.

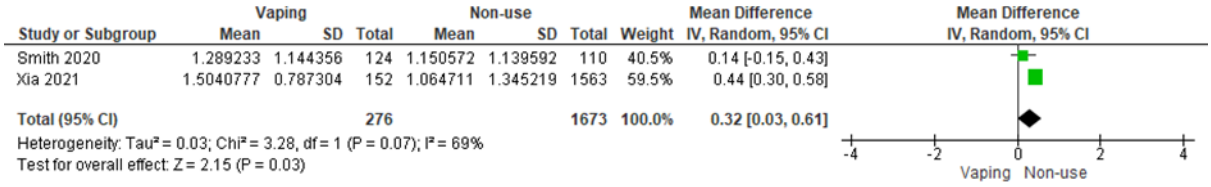
Figure 48. Meta-analysis of cross-sectional studies reporting on urinary NAT levels between vapers and smokers



Four cross-sectional studies compared levels on NAB between vapers and non-users (36, 51, 64, 90). Goniewicz and others reported levels to statistically significantly higher, by approximately 34%, among vapers compared to non-users (36). Smith and others reported levels to be 15% higher, however this was not statistically significant (64). Xia and others reported levels to be 55% higher among vapers, however this was not tested for statistical significance (90). Finally, Shahab and others (51) reported levels to be 38% lower among vapers compared to ex-smokers who use NRT, this however was not statistically significant.

Following the algorithm for selecting studies for meta-analysis (methods: table 6), 2 studies, both measuring levels among daily vapers and non-users, were pooled to assess urinary NAT (52, 90). Across the 2 studies, the pooled geometric mean urinary NAT level was 38% higher among daily vapers than among non-users (LMD= 0.32, 95% CI 0.03, 0.61; p=0.03; figure 49). There was substantial heterogeneity between studies (I^2= 69%). As detailed above, although both studies reported on daily vapers, there were differences in definitions and methodology used in these studies.

Figure 49. Meta-analysis of cross-sectional studies reporting on urinary NAT levels between vapers and non-users



Across cross-sectional studies that measured urinary NAB, vapers' levels were approximately between 2% and 18% and non-users' levels were approximately between 0% and 25% of NAB levels detected among smokers. For urinary NAT, vapers' levels were approximately between 4% and 11% and non-users' levels were approximately between 2% and 9% of NAT levels detected among smokers (figure 51).

Table 28. Cross-sectional studies reporting on levels of anabasine (NAB) and anatabine (NAT) among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
NAB					
Urine biosample					
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**1.422 (1.256-1.61)[c,d] pg/mg creatinine (U)**	**20.85 (18.62-23.34)[c]** **0.068**	**15.67 (14.12-17.39)[a,b]** **0.091**	**1.067 (1.003-1.135)[a]** **1.333**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**1.07 (0.79-1.147)[b,c] pg/mg creatinine (U)**	**6.02 (4.15-8.73)[a,d]** **0.178**	**6.17 (4.31-8.82)[a,d]** **0.173**	**1.52 (1.09-2.12)[b,c]** **0.704**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**1.49 (1.25–1.79)[b,c] pg/mg creatinine (U)**	**4.32 (3.53–5.28)[a,c,d]** **0.345**	**8.11 (6.81–9.64)[a,b,d]** **0.184**	**1.29 (1.06–1.55)[b,c]** **1.155**
Xia et al., 2021, US (90)	See: Xia et al., 2021	1.7 (1.4-1.9) ng/g creatinine (U)	20.5 (18.8-22.2) 0.083	19.6 (18.2-21) 0.087	1.1 (1-1.1) 1.545

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
NAT					
Urine biosample					
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**3.909 (3.402-4.493)[c,d] pg/mg creatinine (U)**	**126.9 (111.7-144.2)[c]** **0.031**	**96.06 (85.66-107.7)[a,b]** **0.041**	**2.921 (2.739-3.114)[a]** **1.338**
Shahab et al., 2017, UK (51)	See: Shahab et al., 2017	**1.79 (1.21-2.67)[b,c] pg/mg creatinine (U)**	**30.8 (18.5-51.1)[a,d]** **0.058**	**32.8 (20.5-52.5)[a,d]** **0.055**	**2.9 (1.81-4.81)[b,c]** **0.617**
Smith et al., 2020, US, UK & Poland (52)	See: Smith et al., 2020	**3.63 (2.96–4.44)[b,c] pg/mg creatinine (U)**	**18.38 (14.64–23.08)[a,c,d]** **0.197**	**34.00 (27.90–41.44)[a,b,d]** **0.107**	**3.16 (2.55–3.91)[b,c]** **1.149**
Xia et al., 2021, US (90)	See: Xia et al., 2021	4.5 (3.8-5.1) ng/g creatinine (U)	130.3 (118.4-142.2) 0.035	125.2 (116.2-134.1) 0.036	2.9 (2.7-3.1) 1.552

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

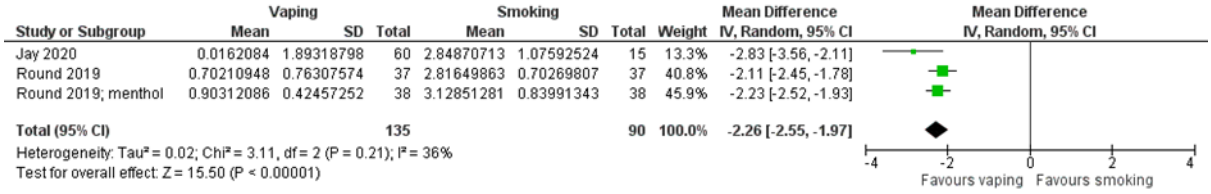
Nornicotine (NNN)

N-Nitrosonornicotine (NNN), a metabolite of nornicotine, always occurs together with NNK, is carcinogenic to humans according to the IARC (79) and is categorised as a carcinogen by the FDA (80). NNN is considered a cause of oral and oesophageal cancer in smokers and particularly in smokeless tobacco users (72).

RCTs

Three RCTs funded by the tobacco industry reported on urinary NNN changes after 5 (7, 74) and 7 days in confinement (8) (table 29). We pooled and meta-analysed data from 2 RCTs where smokers had been randomised to switching from smoking at least 10 cigarettes per day to ad libitum use of a pod vaping product with 50mg/mL nicotine salt e-liquid (74) or to ad libitum use of a cartridge vaping product with 48mg/mL nicotine e-liquid (7) (figure 50).

Figure 50. Meta-analysis of RCTs reporting on urinary NNN levels (nornicotine) after exposure to vaping and smoking



The average urinary NNN levels were statistically significantly lower among vapers than smokers in the 2 RCTs (LMD: -2.26, 95% CI: -2.55, -1.97; 225 participants); the geometric mean NNAL levels were approximately 90% lower among vapers than among smokers (GMR: 0.10, 95% CI: 0.08, 0.14). Heterogeneity between the RCTs was moderate at I^2 = 36%. The third RCT also reported statistically significant reductions in urinary NNN levels 7 days after switching from smoking to ad libitum vaping product use with 4.3mg/mL nicotine e-liquid (~77% reduction), ad libitum HTP use (~54% reduction) and abstinence from tobacco or nicotine products (~80% reduction).

Table 29. Randomised controlled trials, cross-over and longitudinal studies reporting on nornicotine (NNN) levels among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Urine biosample							
RCT							
Jay et al., 2020, US (74)	5 days (A)	See: Jay et al., 2020	n=60 6.1 (36.1) ng/24h (U) ↓**61.4%**	NA	n=15 30.8 (45.5) ↑86.7%	n=11 0.2 (0.2) ↓**98.9%**	NA
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 2.6[c,e] ng/24h (U) ↓**76.5%**	NA	n=30 10.85[a,d,e] ↓11.3%	n=29 2.49[c,e] ↓**79.8%**	n=28 6.1[c,d] ↓**53.5%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 2.7 (2.4) ng/24h (U) ↓**87.4%** Menthol, n=38: 2.7 (1.2) ng/24h (U) ↓**91.7%**	NA	NA	NA	Non-menthol, n=38: 3.2 (4.9) ↓**88.5%** Menthol, n=40: 2.5 (1.2) ↓**89.8%**

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

Three cross-sectional studies reported levels of NNN among vapers and smokers. (29, 36, 90) (table 30). Goniewicz and others reported urinary NNN levels to be statistically significantly lower among vapers, by approximately 71%, in comparison to smokers (36). Xia and others reported urinary NNN levels to be around 62% lower among daily vapers compared to daily smokers, and Bustamante and others reported urinary NNN levels to be on average 99% lower and salivary NNN levels to be on average 85% lower among vapers compared to smokers, comparisons were not tested for statistical significance (29, 90).

Four studies reported comparisons of NNN levels between vapers and non-users (29, 36, 55, 90), however, following the algorithm (methods: table 6), data from these studies could not be pooled for meta-analysis. Goniewicz and others reported statistically significant differences between the groups, with vapers having 81% higher levels in urinary samples compared to non-users (36). Bustamante and others reported no difference between urinary levels, however reported salivary NNN levels to be on average 58 times higher among vapers compared to non-smokers; however, the reported variation was wide, sample size was small, and differences were not tested for statistical significance (29). Xia and others reported levels to be 2.6 times higher among daily vapers compared to never smokers, and Dai and others reported levels to be 50% higher among vapers with respiratory symptoms than non-users with symptoms and 30% higher among vapers with respiratory symptoms compared with non-users without symptoms, although neither study tested the comparisons for statistical significance (55, 90).

Across cross-sectional studies that measured urinary NNN, vapers' levels were approximately between 15% and 38% and non-users' levels were approximately between 0% and 16% of NNN levels detected among smokers (figure 51).

Table 30. Cross-sectional studies reporting on nornicotine (NNN) levels among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
Urine biosample					
Bustamante et al., 2018, US (29)	See: Bustamante et al., 2018	14.6 (23.1) pg/mL (S) 0.001 (0.002) pmol/mL (U)	NA	94.5 (176) (S) 0.154 0.16 (0.5) (U) 0.006	0.25 (0.28) (S) 58.4 0.001 (0.001) (U) 1.0
Dai et al., 2020, US (55)	See: Dai et al., 2020	Without symptoms: 3.3 (2.8-3.8) pg/mg creatinine (U) With symptoms: 3.6 (2.7-4.8) pg/mg creatinine (U)	8 (6.9-9.4) 0.413 10.2 (9.4-11.2) 0.353	NA	2.2 (2-2.3) 1.5 2.8 (2.5-3.0) 1.286

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**3.471 (3.033-3.972)[c,d] pg/mg creatinine (U)**	**11.78 (10.66-13.01)** **0.295**	**11.80 (10.84-12.85)[a]** **0.294**	**1.923 (1.81-2.043)[a]** **1.805**
Xia et al., 2021, US (90)	See: Xia et al., 2021	5.2 (4.3-6) ng/g creatinine (U)	12.9 (12.1-13.8) 0.403	13.8 (13-14.6) 0.377	2 (1.9-2.1) 2.6

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

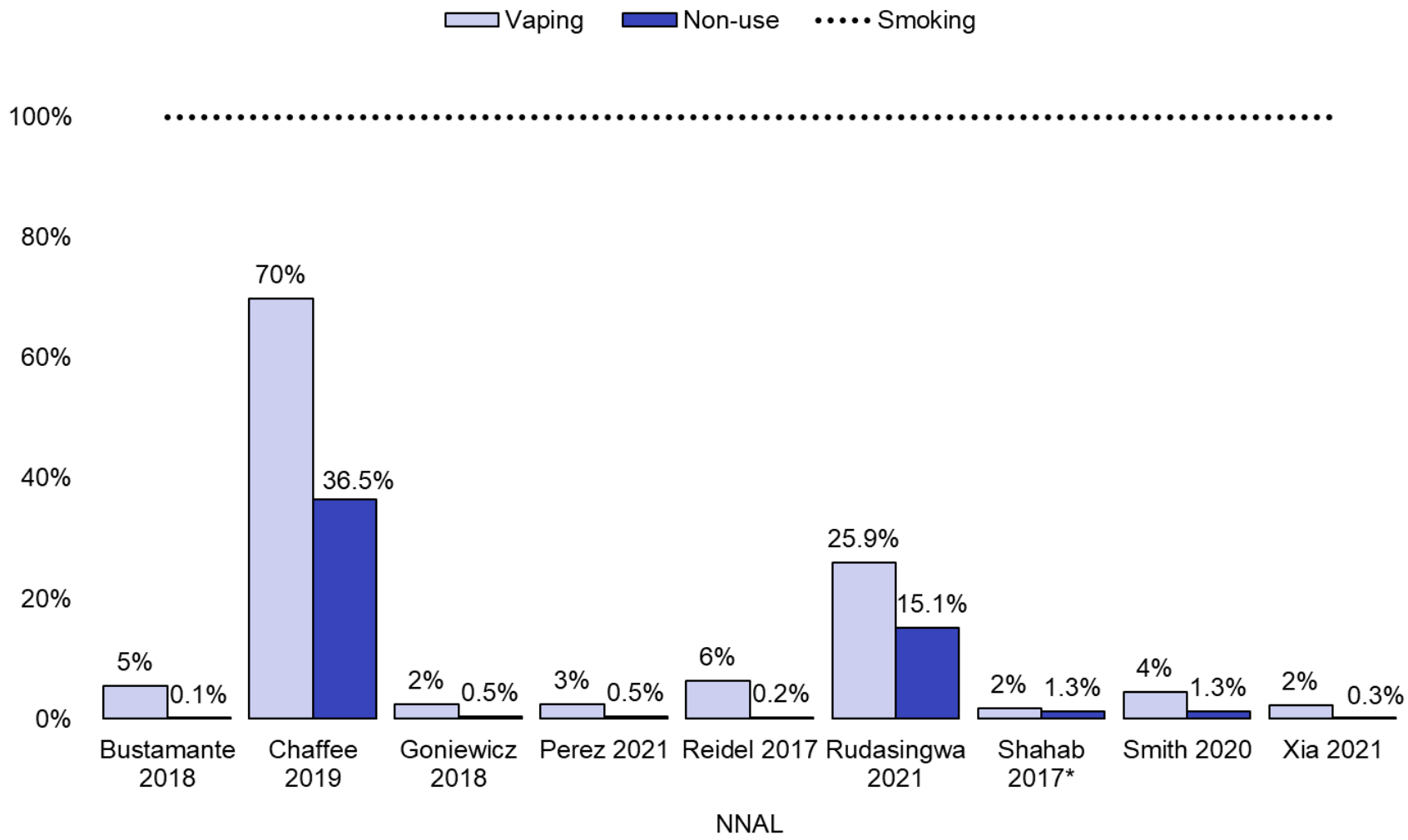
[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

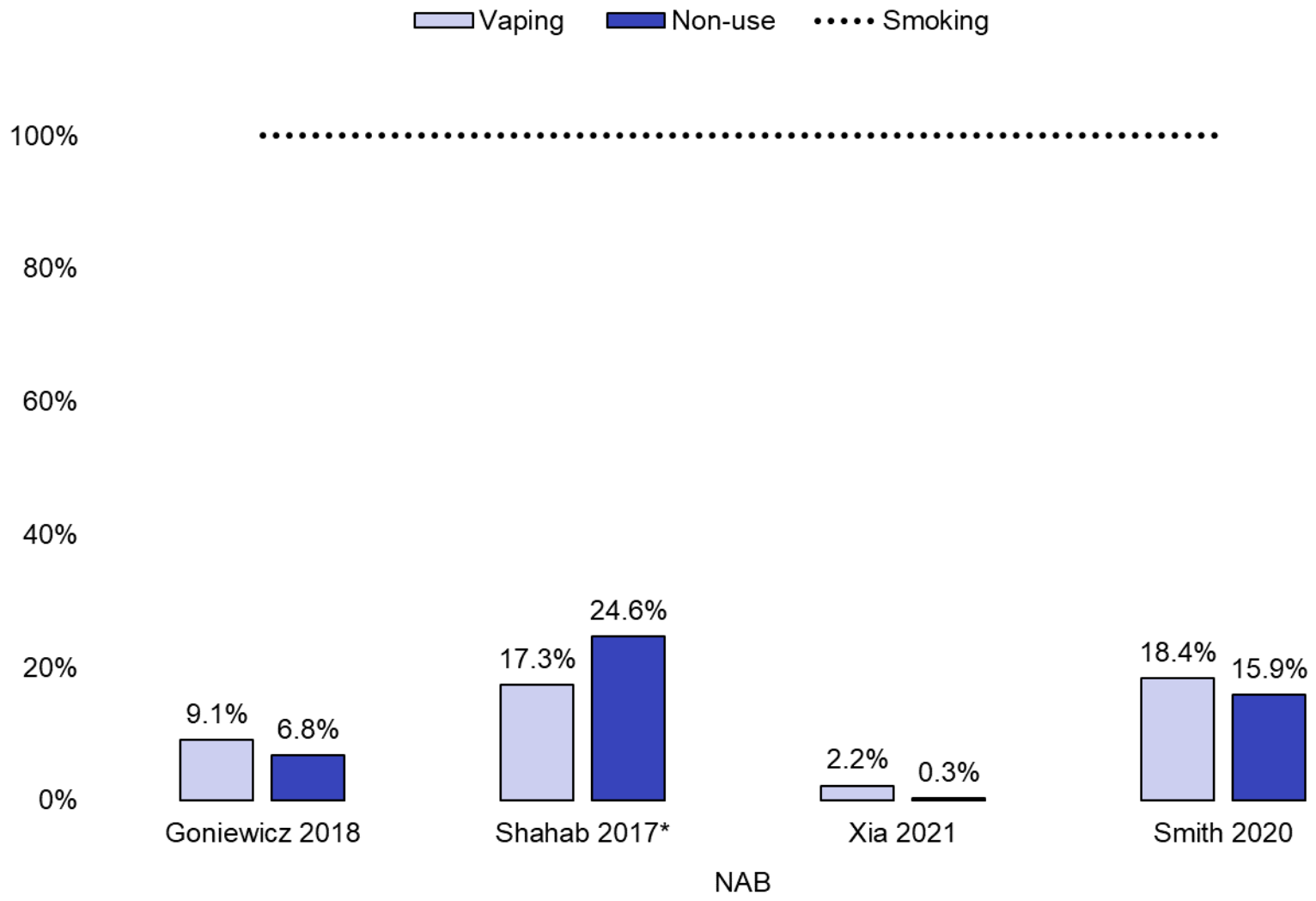
Figure 51. Levels of urinary biomarkers of tobacco-specific nitrosamines in vapers and non-users relative to smokers

Levels of urinary biomarkers of NNAL in vapers and non-users relative to smokers



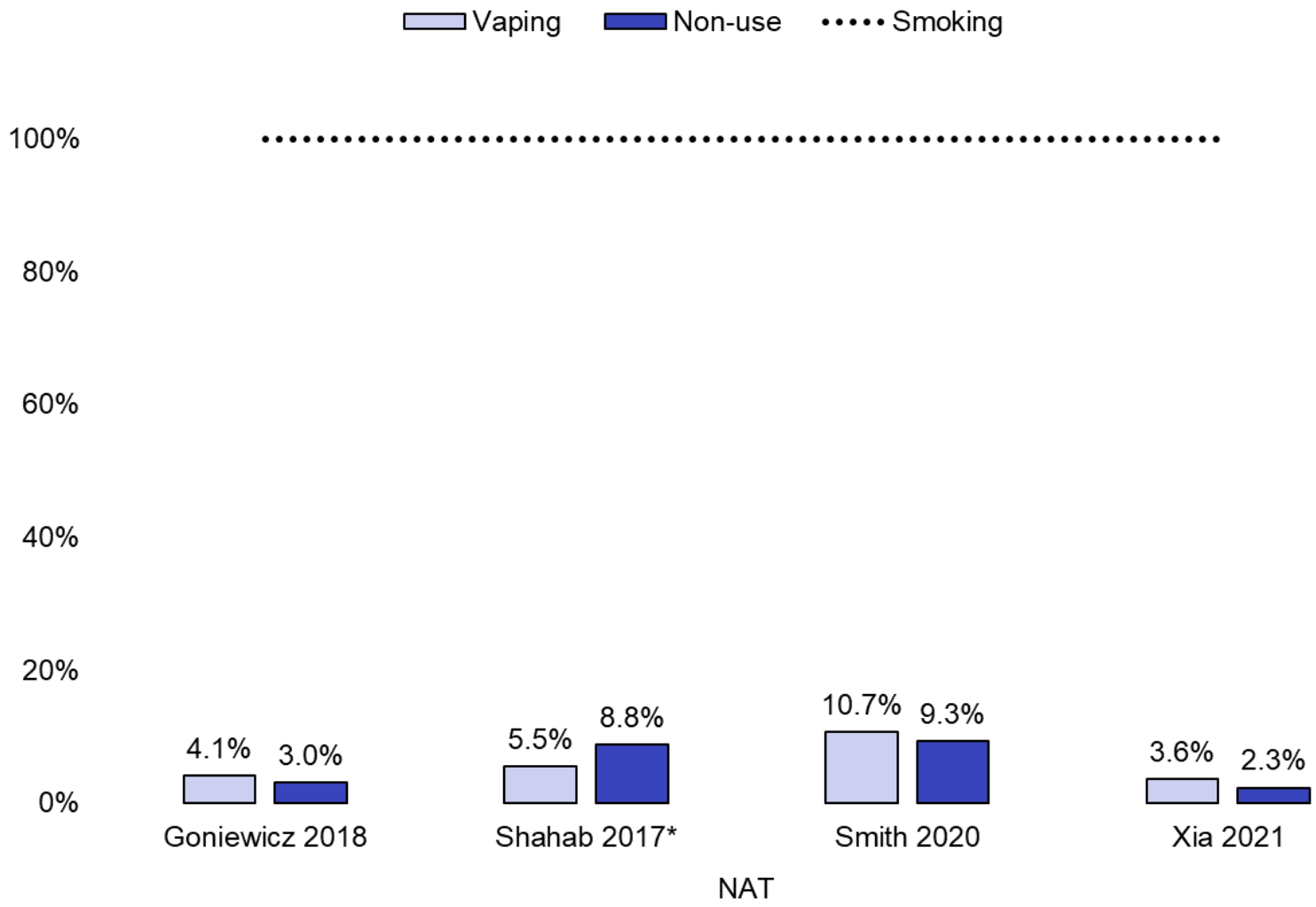
Note: * Non-users in Shahab et al. (51) were all using NRT.

Levels of urinary biomarkers of NAB in vapers and non-users relative to smokers



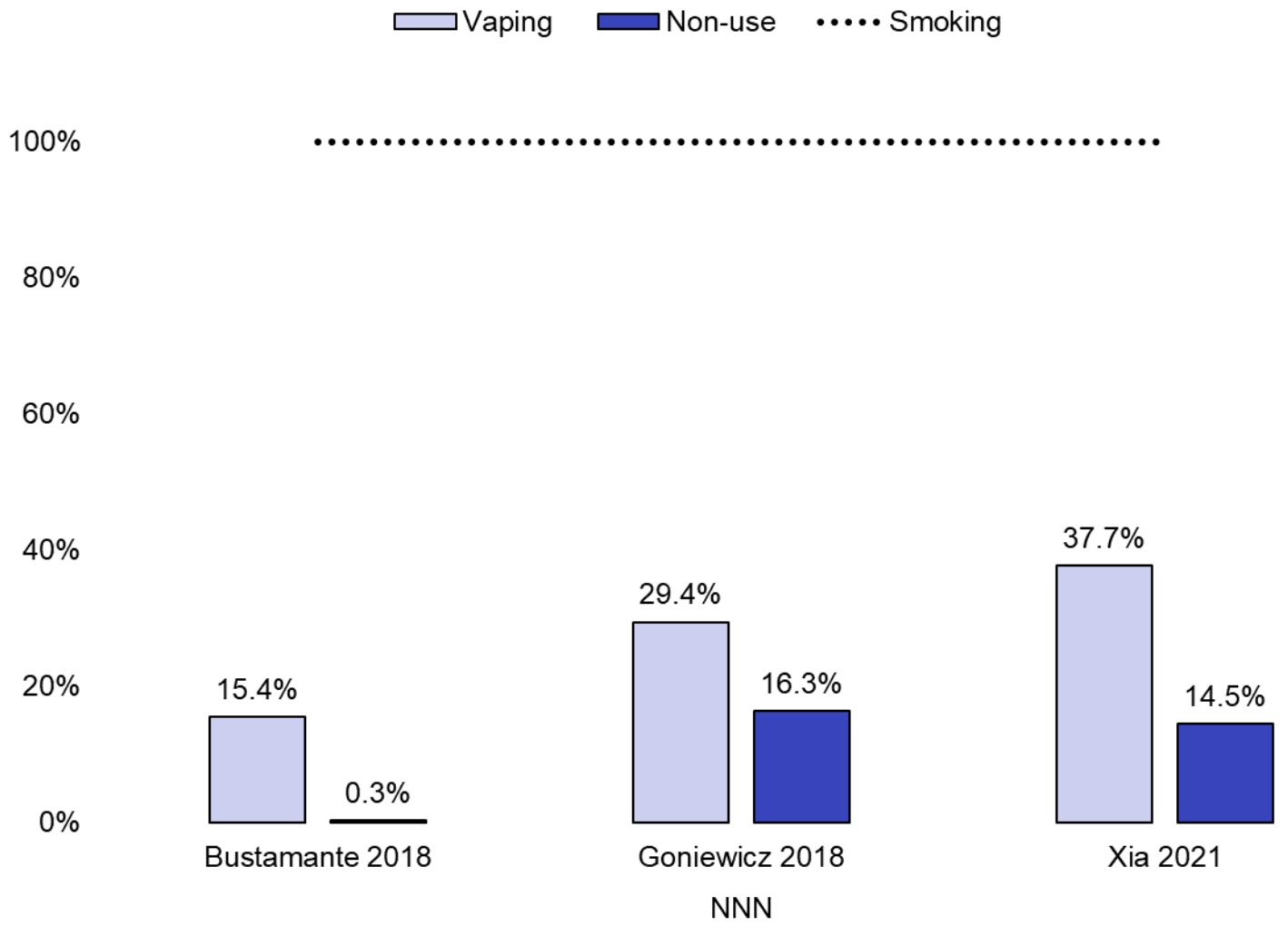
Note: * Non-users in Shahab et al. (51) were all using NRT.

Levels of urinary biomarkers of NAT in vapers and non-users relative to smokers



Note: * Non-users in Shahab et al. (51) were all using NRT.

Levels of urinary biomarkers of NNN in vapers and non-users relative to smokers



Summary of studies reporting on exposure to tobacco-specific nitrosamines

Findings of the included studies agree that exposure levels to tobacco-specific nitrosamines are reduced substantially after smokers or dual users switch to vaping only. Provided that follow-up length after switching is of long enough duration (in the case of NNAL with a half-life of over 10 days), observed average reductions in biomarker levels of tobacco-specific nitrosamines, may result in levels which are similar to those measured in non-users.

The findings from cross sectional studies appear to support the longitudinal studies. Overall, levels of tobacco-specific nitrosamines appear to be substantially lower among vapers compared to smokers, with publications often citing 90% or more reduction in exposure. When compared to non-users, the evidence is less clear. Pooled analysis suggests that levels of tobacco-specific nitrosamines are significantly higher among vapers compared to non-users. However, limitations of the cross-sectional design should be considered when interpreting these findings. For the one study that examined vapers with and without respiratory symptoms compared with non-users with and without symptoms, levels in vapers were higher than among non-users for both groups although these were not tested for statistical significance.

Two studies reported on levels of NNAL among adolescents (32, 50). The difference between vapers and smokers was substantially less among adolescents compared to adult studies. However, this is likely due to heavier vaping and smoking patterns among adults, with adolescent smokers and vapers only having to report a single use in the past 7 days to participate in the study (32). Differences between vapers and non-users were broadly the same as adult samples.

Furthermore, it is important to note that although tobacco-specific nitrosamine levels are suggested to be higher among vapers than non-users, this is substantially smaller difference than that between smokers and non-users.

7.6 Biomarkers of exposure to other potential toxicants

Next, we discuss studies that reported on exposure to other potential toxicants from the WHO list of priority toxic contents and emissions of tobacco products, including polyaromatic hydrocarbons (benzo[a]pyrene and pyrene), aromatic amines (1-Aminonaphthalene, 2-Aminonaphthalene, 3 Aminobiphenyl and 4-Aminobiphenyl) and other compounds (methods: table 3). No longitudinal study reported on associations of vaping with biomarkers of acetone, ammonia, m-Cresol, p-Cresol, catechol, phenol, pyridine, resorcinol, toluene or quinoline. A likely reason for the lack of information on these biomarkers is that most of them do not have established metabolites in humans.

Study characteristics

The literature search identified 3 studies reporting on 2 RCTs (7, 8, 25), one cross-over trial (11), one non-randomised longitudinal study (23) and 5 cross sectional studies (36, 47, 54, 66, 91) reporting on other potential toxicants.

Of the 5 studies with more than one time-point reporting on biomarkers of other potential toxicants, one study reported in 2 publications was conducted in the US (7, 25), one in the UK (8), one in Canada (11) and one in Poland (23). Two RCTs were funded by the tobacco industry (7, 8, 25) (appendices: table 5).

All 4 cross-sectional studies investigating PAHs were from the US and used data from Wave 1 of the PATH study (36, 47, 54, 66). One cross-sectional study from the US reported on urinary levels of 2-Aminonaphtalene (91).

Sample sizes of the included studies ranged from 20 in a longitudinal study (23) to 158 in an RCT (7, 25), participants' mean age ranged from 31 (23) to 42.6 (7, 25), and between 29.2% (11) and 60% (23) of participants in the included studies were women. All longitudinal studies explored exposure to other potential toxicants in participants from the general population.

Sample sizes of the cross-sectional PAH studies ranged from 1,857 (47) to 8,327 (66). One study included women of a reproductive age (47) and another of pregnant and non-pregnant women (54); 54% to 60% of the participants in the other 2 were women (36, 66). Fuller and others explored exposure to 2-Aminonaphtalene in 23 participants (91).

RCTs

A total of 306 participants were recruited in 2 RCTs (7, 8, 25). Both RCTs were conducted in confinement for 5 (7, 25) and 7 days (8), and recruited smokers who smoked at least 10 cigarettes per day. The RCTs randomised participants to vaping, smoking, HTP use, nicotine gum use or no use of tobacco and nicotine products.

Cross-over studies

A cross-over study (11) recruited 48 dual users who smoked at least 5 cigarettes per day and included 7-day cross-over conditions of ad libitum vaping, smoking and no use of tobacco or nicotine products. Adherence to vaping only and non-use conditions could not be enforced.

Longitudinal studies

A non-randomised longitudinal study (23) recruited 20 participants who smoked at least 5 cigarettes per day, asked them to switch to using a cartridge vaping product and followed them up for 2 weeks. Adherence to vaping could not be enforced, therefore groups of

vapers and those who concurrently used the assigned vaping product and continued smoking were compared at the follow-up.

Cross-sectional studies

All 4 cross-sectional PAH studies defined vaping as every day or someday use (36, 47, 54, 66). 2 defined non-use as those who had never used tobacco products (47, 66) and one did not report definitions of non-use (36).

For the one cross-sectional study which reported on exposure to aromatic amines, the frequency of vaping required for participation was not reported, and non-users were defined as those who had not used tobacco or vaping products for 6 months (91).

Risk of bias in included studies

RCTs

The 2 RCTs (7, 8, 25) were assessed to have some concerns in terms of overall risk of bias according to the RoB2 risk of bias tool (appendices: table 1), with lack of information on the randomisation process and pre-specified data analysis plans.

Cross-over studies

A cross-over study (11) was assessed to be at high risk of bias due to potential deviations from intended interventions (adherence to vaping only and non-use conditions was not enforced) according to the RoB2 risk of bias tool for cross over studies (appendices: table 2).

Longitudinal studies

A non-randomised longitudinal study (23) was assessed at moderate risk of bias due to potential that participants continued smoking during the follow-up period according to the ROBINS-I risk of bias tool (appendices: table 3).

Cross-sectional studies

Quality of cross-sectional studies that explored exposure to PAH was assessed using Biocross quality appraisal tool and is reported in the appendices (table 4). Studies scored between 12 (54) and 16 (47) out of a maximum score of 20, with most studies of reasonably good quality. The main limitations were associated with a lack of detail about statistical adjustments for confounders and limited detail on laboratory measurement procedures (for example, blinded analyses, reporting on quality control procedures).

The study that reported exposure to aromatic amines scored 9 out of a possible 20 marks (91). The main limitations were associated with a lack of clear definition of vaping, lack of

adjustments for cofounding variables, as well as a lack of discussion on limitations arising from the cross-sectional study design.

Study findings: polyaromatic hydrocarbons

Benzo[a]pyrene (3-OH-B[a]P) and pyrene (1-HOP)

Benzo[a]pyrene and pyrene are known polyaromatic hydrocarbons (PAHs) that form from the incomplete combustion of organic compounds, such as tobacco smoke. However, smokeless tobacco products also contain PAHs due to the tobacco curing process. PAHs are not specific to tobacco products and occur in foods and air pollution. Previously, PAHs have also been identified in e-liquids and vaping product aerosols although generally at very low levels (3).

Benzo[a]pyrene is carcinogenic to humans according to the IARC (79) and is categorised as a carcinogen by the FDA (80). Day-to-day human exposure to benzo[a]pyrene has been studied for years, with coal-processing waste products, petroleum sludge, asphalt and tobacco smoking known as sources containing high levels of the carcinogen (92). The metabolite of benzo[a]pyrene is 3-hydroxy-benzo[a]pyrene (3-OH-B[a]P).

Pyrene is classified as not carcinogenic to humans (79) but is always a component of mixtures of other polyaromatic hydrocarbons that are carcinogenic (93). Therefore, pyrene’s urinary metabolite 1-hydroxypyrene (1 HOP) is simultaneously considered an accepted biomarker of carcinogenic polyaromatic hydrocarbons dose (93).

RCTs

A single RCT reported change in urinary 3-OH-B[a]P in smokers who had switched to ad libitum cartridge vaping product use with 48mg/mL nicotine e-liquid in confinement for 5 days (7) (table 31). Both menthol and non-menthol flavoured vaping product user groups showed a statistically significant reduction by over 60% in urinary 3-OH-B[a]P at 5-day follow-up compared with baseline. Round and others (7) and another RCT conducted in confinement for 7 days (8) reported statistically significant changes in urinary 1-HOP levels after switching from smoking to using vaping product; both trials on average reported over 60% reduction in urinary 1-HOP levels among vaping groups at 5- and 7-day follow-ups. McEwan and others also randomised participants to a nicotine cessation group—these participants on average demonstrated a statistically significant 78% reduction in 1-HOP levels at the follow-up; 1-HOP levels in the non-user group were statistically significantly lower than in the vaper group at follow-up (8).

Cross-over studies

A cross-over study (11) reported a statistically significant reduction by over 30% in urinary 1-HOP levels after 7 days vaping only (table 31). Authors also noted statistically significantly lower 1-HOP levels when participants exclusively vaped compared with when

they concurrently vaped and smoked, which suggests reduced exposure to polyaromatic hydrocarbons when dual users abstain from smoking for a week.

Longitudinal studies

A single non-randomised longitudinal study (23) did not find a statistically significant change in urinary 1-HOP levels 2 weeks after switching from smoking to exclusive vaping product use (table 31).

Table 31. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of polyaromatic hydrocarbons among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
3-OH-B[a]P (Benzo[a]pyrene)							
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 93.6 (72.6) pg/24h (U) ↓**63.7%** Menthol, n=38: 81.1 (52.9) pg/24h (U) ↓**70.0%**	NA	NA	NA	Non-menthol, n=38: 133.4 (171.6) ↓78.5% Menthol, n=40: 104.5 (110) ↓**44.9%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
1-HOP (Pyrene)							
Urine biosample							
RCT							
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 97.43[c,d] ng/24h (U) ↓**65.9%**	NA	n=30 313.33[a,d,e] ↑1.2%	n=29 80.05[a,c,e] ↓**78.0%**	n=28 106.71[c,d] ↓**62.5%**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 183.9 (128.5) ng/24h (U) ↓**63.5%** Menthol, n=38: 186.5 (180.6) ng/24h (U) ↓**67.2%**	NA	NA	NA	Non-menthol, n=38: 336.1 (731) ↓**50.5%** Menthol, n=40: 199.9 (158.9) ↓**62.2%**
Cross-over							
Czoli et al., 2019, Canada (11)	7 days (A)	See: Czoli et al., 2019	n=48 141.1	n=48 203.3	n=48 249.23	n=48 175.1	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			(98.3-202.5)[b,c] pg/mg creatinine (U) ↓**30.6%**	(153.9-268.7)[a] Baseline	(197.2-315.1)[a,b,d] ↑**22.6%**	(134.3-228.2) ↓13.9%	
Longitudinal							
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 746 ng/g creatinine (U) ↓4.1%	n=11 617 ↓18.1%	NA	NA	NA

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

Four studies reported on urinary levels of 1-HOP between vapers and smokers (36, 47, 54, 66) (table 32). Three of these studies used subsets of the same PATH survey data from wave 1, therefore following the algorithm (methods: table 6), data could not be pooled for meta-analysis. Two reported that vapers had statistically significantly lower levels, by approximately 33% (47) to 47% (36), when compared to smokers. Wang and others reported levels to be 48% lower among vapers compared to smokers, and Coleman and others reported levels to be around 38% lower among non-pregnant women who vape and 34% lower among pregnant women who vape compared to non-pregnant women and pregnant women who smoke respectively, but neither comparison was tested for statistical significance (54, 66).

Three studies reported differences in urinary 1-HOP between vapers and non-users (36, 47, 66). Goniewicz and others found statistically significantly higher levels of urinary 1-HOP among vapers compared to non-users, by on average 26% (36). Perez and others reported levels to be 2 times higher among vapers compared to non-users, however this was not statistically significant (47). Wang and others (2018) also reported that levels of 1-HOP were 16% higher among vapers compared to non-users, however this was not tested for statistical significance (66).

Across cross-sectional studies that measured urinary 1-HOP, vapers' levels were approximately between 52% and 67% and non-users' levels were approximately between 33% and 44% of 1-HOP levels detected among smokers (figure 52).

Table 32. Cross-sectional studies reporting on levels of biomarkers of polyaromatic hydrocarbons among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
1-Hydroxypyrene (1-HOP)					
Urine biosample					
Coleman et al., 2021, US (54)	See: Coleman et al., 2021	Non-pregnant: 0.18 (0.14-0.23) ng/mg creatinine (U) Pregnant: 0.25 (0.14-0.43) ng/mg creatinine (U)	0.34 (0.3-0.39) 0.529 0.37 (0.24-0.55) 0.676	0.29 (0.27-0.32) 0.621 0.38 (0.32-0.45) 0.658	NA
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**0.161 (0.143-0.181)[c,d] ng/mg creatinine (U)**	**0.355 (0.339-0.373)[c]** **0.454**	**0.303 (0.287-0.321)[a,b]** **0.531**	**0.128 (0.121-0.136)[a]** **1.258**
Perez et al., 2021, US (47)	See: Perez et al., 2021	**0.2 (0.1-0.2)[c] ng/mg creatinine (U)**	NA	**0.3 (0.3-0.4)[a,d]** **0.667**	**0.1 (0.1-0.2)[c]** **2.0**
Wang et al., 2018, US (66)	n = 8327 % within age groups: 18-24—15.8%, 25-34—19.7%, 35-54—36.7%, >54—27.8%, 54.4%	159 (146-174) ng/L (U)	NA	306 (151-199)	136 (129-144)

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
	females, 60.6% non-Hispanic White, 14.7% non-Hispanic Black, 7.7% Hispanic, 17.0% Asian and other ethnicity. Vapers (n=280): use VP everyday or some days. Smokers (n=3964): smoked >100 TC, smoke everyday or some days. Non-users (n=1700): has never used tobacco products.			0.520	1.169

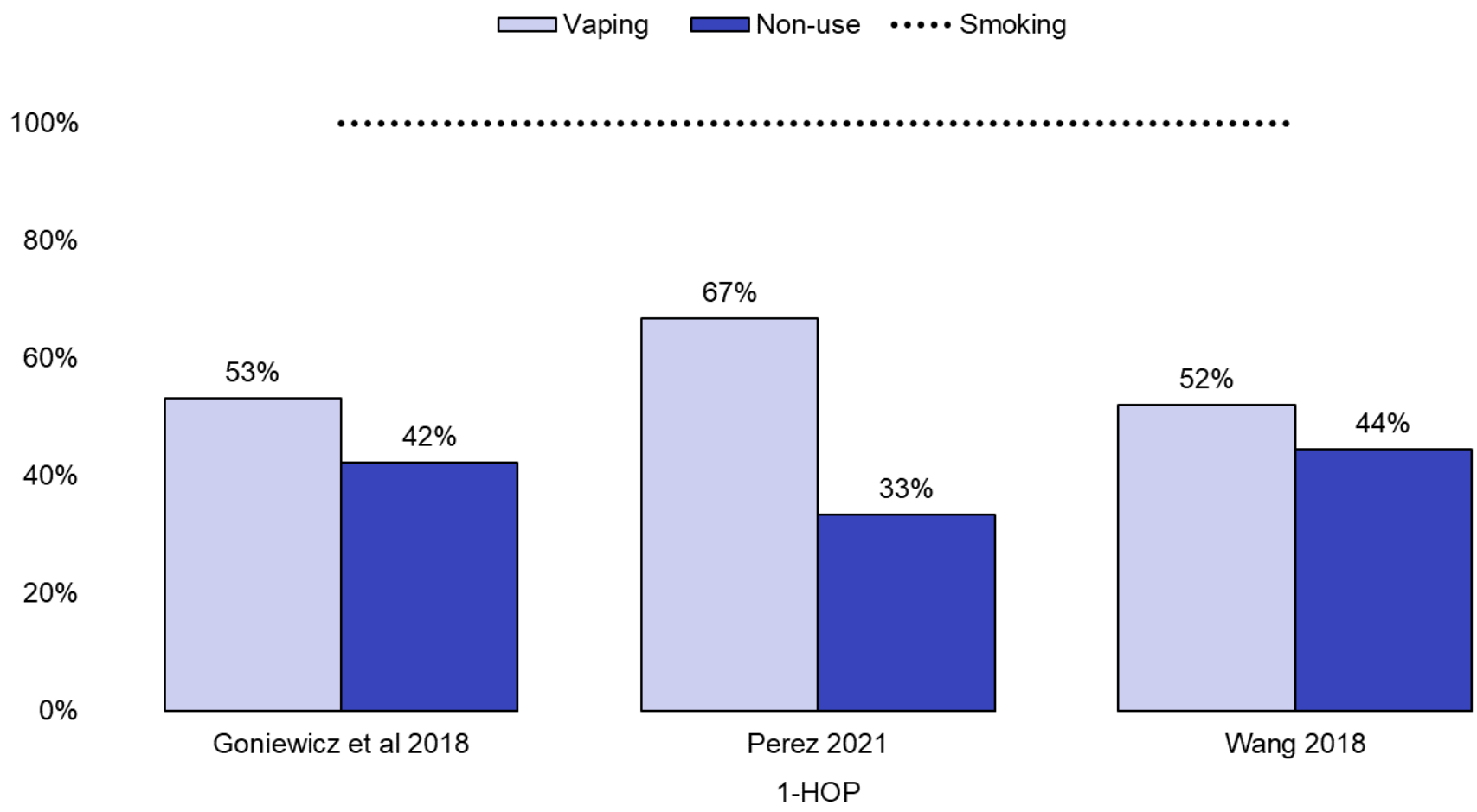
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 52. Levels of urinary 1-HOP in vapers and non-users relative to smokers



Summary of studies reporting on exposure to polyaromatic hydrocarbons

Based on findings from 4 studies with more than one time point, exposure to polyaromatic hydrocarbons appears to be reduced significantly, by around 60%, after switching from smoking to vaping product use for at least 5 days. However, this reduction was achieved in RCTs conducted in confinement—ad libitum use studies in real-world settings suggest a smaller reduction in exposure to polyaromatic hydrocarbons after a week or no change after 2 weeks. As there are other environmental sources of polyaromatic hydrocarbons apart from smoking, such as coal and wood fires and motor vehicles, RCTs conducted in confinement might best represent relative reductions in exposure after switching from smoking to vaping product use. Only one RCT reported lower exposure to polyaromatic hydrocarbons among non-users compared with vapers 7 days after randomisation.

Overall, cross-sectional studies suggest that urinary 1-HOP is significantly lower among vapers compared to smokers. Levels were reported to be higher among vapers compared to non-users, however findings were not consistent, and participants' past tobacco use may be contributing to some of these study findings.

Study findings: aromatic amines

1-Aminonaphthalene (1-AN), 2-Aminonaphthalene (2-AN), 3-Aminobiphenyl (3-ABP), 4-Aminobiphenyl (4-ABP)

Four aromatic amines are included in the WHO priority toxic contents and emissions list (94). 1-Aminonaphthalene (1-AN) is considered not carcinogenic to humans while 2-Aminonaphthalene (2-AN) and 4-Aminobiphenyl (4-ABP) are considered carcinogenic to humans by the IARC (79). According to the FDA, 3 aromatic amines from the WHO list—1-Aminonaphthalene (1-AN), 2-Aminonaphthalene (2-AN) and 4-Aminobiphenyl (4 ABP)—are recognised carcinogens (80). Aromatic amines are formed during tobacco pyrolysis, and bladder cancer is one of the risks associated with exposure to aromatic amines in tobacco smoke (95).

RCTs

Two RCTs funded by the tobacco industry reported on changes in urinary levels of aromatic amines after switching to vaping product use for 5 (7) and 7 days (8) (table 33). Round and others reported statistically significant reductions in levels of 4 aromatic amines among smokers who switched to vaping product use for 5 days, with the approximate size of reduction ranging from 63% for 4-ABP to 96% for 1-AN. McEwan and others reported statistically significant reductions in urinary 2-AN (~92.5%) and 4-ABP levels (~87.7%) 7 days after switching from smoking to a cartridge vaping product. Additionally, exposure to these 2 aromatic amines also decreased statistically significantly by the same magnitude in participants who were randomised to HTP use or no use of tobacco products for 7 days (8).

Table 33. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of aromatic amines among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
1-Aminonaphthalene (1-AN)							
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 4.5 (2.6) ng/24h (U) ↓**95.9%** Menthol, n=38: 5.3 (2.6) ng/24h (U) ↓**95.0%**	NA	NA	NA	Non-menthol, n=38: 4.3 (1.7) ↓**95.8%** Menthol, n=40: 7.4 (13.5) ↓**93.2%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
2-Aminonaphthalene (2-AN)							
Urine biosample							
RCT							
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 2.38[c] ng/24h (U) ↓**92.5%**	NA	n=30 32.38[a,d,e] ↑2.7%	n=29 2.4[c] ↓**92.5%**	n=28 3.03[c] ↓**90.1%**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 2.6 (1.4) ng/24h (U) ↓**90.5%** Menthol, n=38: 2.5 (1.4) ng/24h (U) ↓**91.4%**	NA	NA	NA	Non-menthol, n=38: 2.5 (1.4) ↓**91.0%** Menthol, n=40: 2.5 (1.4) ↓**91.5%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
3-Aminobiphenyl (3-ABP)							
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 2.8 (1.4) ng/24h (U) ↓**73.6%** Menthol, n=38: 2.2 (1.0) ng/24h (U) ↓**78.2%**	NA	NA	NA	Non-menthol, n=38: 2.1 (1.3) ↓**78.1%** Menthol, n=40: 2.0 (0.9) ↓**80.8%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
4-Aminobiphenyl (4-ABP)							
Urine biosample							
RCT							
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 2.61[c] ng/24h (U) ↓**87.7%**	NA	n=30 22.36[a,d,e] ↑4.9%	n=29 2.83[c] ↓**87.2%**	n=28 3.36[c] ↓**84.6%**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 7.8 (3.4) ng/24h (U) ↓**63.2%** Menthol, n=38: 6.1 (2.6) ng/24h (U) ↓**72.8%**	NA	NA	NA	Non-menthol, n=38: 7.2 (3.8) ↓**68.1%** Menthol, n=40: 6.6 (2.3) ↓**71.3%**

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

In Fuller and others study, vapers were reported to have statistically significantly higher levels of 2-Aminonaphtalene, by approximately 29%, compared to non-users, however these findings were based on a very small sample (91) (table 34).

Table 34. Cross-sectional studies reporting on levels of biomarkers of aromatic amines among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	**Dual users[b]**	**Smoking[c]**	**Non-users[d]**
2-AN (2-Aminonaphtalene)					
Urine biosample					
Fuller et al., 2018, US (91)	n = 23 Vapers' mean (SD) age: 39.4 (13.5), 30.8% females; non-users' mean (SD) age: 30.1 (7.7), 50.0% females. Vapers (n=13): NR. Non-users (n=10): self-reported non-use of tobacco or nicotine products for past 6 months.	**1.46 (0.23)[d] ng/mL (U)**	NA	NA	**1.13 (0.36)[a]** **1.292**

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Summary of studies reporting on exposure to aromatic amines

Evidence regarding change in exposure to aromatic amines when smokers switch to vaping product use is limited to 2 tobacco-industry-funded RCTs conducted in confinement for up to a week and one small cross-sectional study. Findings from the trials indicate that switching from smoking to vaping product use for a week might significantly reduce approximate exposure to aromatic amines by over 60%, and that the magnitude of exposure reduction is similar between vapers, HTP users and non-users. The initial results were achieved in established smokers with short follow-up periods.

Study findings: other potential toxicants

Ortho-Toluidine (o-Tol)

Ortho-Toluidine is carcinogenic to humans according to the IARC (79) and is categorised as a carcinogen by the FDA (80). It is a known bladder carcinogen in humans (96).

RCTs

Two RCTs funded by the tobacco industry reported on changes in urinary o-Toluidine levels after switching from smoking at least 10 cigarettes per day to vaping product use for 5 (7) or 7 days (8) (table 35). Both RCTs reported statistically significant reductions in o-Toluidine levels—the reductions ranged from approximately 55% at day 5 (7) to 79% at day 7 (8) among vaping product users compared with baseline. McEwan and others further reported that average o-Toluidine levels at 7-day follow-up in vapers were statistically significantly lower than in smokers and non-users. The authors explained that the lower urinary o-Toluidine levels in vapers compared with non-users were due to the ‘inexplicably high value for o-Toluidine on Day 6–7 of the study’ in one participant from the nicotine cessation group (8).

In summary, based on findings from the 2 RCTs, o-Toluidine levels seemed to decrease after switching from smoking to vaping product use in confinement for up to a week, but evidence regarding exposure difference to o-Toluidine between vapers and non-users is not clear.

Hydrogen cyanide (Thiocyanate)

Hydrogen cyanide is categorised as a respiratory and cardiovascular toxicant by the FDA (80). Thiocyanate, the metabolite of hydrogen cyanide, was the first biomarker of smoking dose (97). However, there are other sources for cyanide apart from smoking, therefore normal levels of cyanide in humans have not been defined yet, and levels among smokers and non-smokers often overlap (97, 98).

RCTs

One tobacco-industry-funded RCT reported on urinary thiocyanate level changes 5 days after switching from smoking at least 10 cigarettes per day to ad libitum use of a cartridge vaping product with 48mg/mL nicotine, tobacco- or menthol-flavoured e-liquid (7) (table 35). The trial reported statistically significant reductions in thiocyanate levels after 5 days of tobacco- or menthol-flavoured vaping product use that ranged from 36.0% to 39.4% compared with baseline. Slightly smaller but statistically significant reductions in thiocyanate levels by approximately 29% were reported for smokers who switched to use of 4 mg nicotine gum for 5 days. As study authors noted, the half-life of thiocyanate is longer than the follow-up used in this RCT (1-2 weeks (97)), therefore larger reductions in urinary thiocyanate levels might be expected after longer periods of switching from smoking to vaping product use.

Ortho-Cresol (o-Cresol)

Ortho-Cresol is categorised as a carcinogen and respiratory toxicant by the FDA (80). o-Cresol sulfate is used as a metabolite of exposure to ortho-Cresol.

RCTs

One tobacco-industry-funded RCT reported on urinary and blood plasma o-Cresol sulfate level changes 5 days after switching from smoking at least 10 cigarettes per day to ad libitum use of a cartridge vaping product with 48mg/mL nicotine, tobacco- or menthol-flavoured e-liquid (25) (table 35). The study reported statistically significant reductions in both urinary and blood plasma o-Cresol sulfate levels by over 80% compared with baseline.

Table 35. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of biomarkers of other potential toxicants among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
o-Toluidine							
Urine biosample							
RCT							
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 30.69[c,d] ng/24h (U) ↓**79.0%**	NA	n=30 146.6[a,d,e] ↓5.2%	n=29 61.57[a,c,e] ↓**61.8%**	n=28 38.4[c,d] ↓**75.4%**
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 109 (108.4) ng/24h (U) ↓**58.0%** Menthol, n=38: 90.2 (33.3) ng/24h (U) ↓**55.7%**	NA	NA	NA	Non-menthol, n=38: 97.8 (75.1) ↓**51.9%** Menthol, n=40: 96 (44.7) ↓**52.7%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Thiocyanate (Hydrogen cyanide)							
Urine biosample							
RCT							
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 103.8 (45.7) µmol/24h (U) ↓**39.4%** Menthol, n=38: 119.5 (54.5) µmol/24h (U) ↓**36.0%**	NA	NA	NA	Non-menthol, n=38: 101.6 (46.8) ↓**29.3%** Menthol, n=40: 108.5 (48) ↓**29.0%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
o-Cresol							
RCT							
Urine and blood biosample							
Liu et al., 2020, US (25)	5 days (A)	See: Liu et al., 2020	Non-menthol, n=37: % change from baseline (U): ↓**80.1%** % change from baseline (BP): ↓**81.4%** Menthol, n=38: % change from baseline (U): ↓**84.8%** % change from baseline (BP): ↓**87.8%**	NA	NA	NA	NR

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Summary of studies reporting on exposure to other toxicants

We identified 2 tobacco-industry-funded RCTs that reported in 3 publications on changes associated with vaping product use in 3 metabolites of other potential toxicants from the WHO priority toxic contents and emissions list (94). The evidence from the 2 RCTs in confinement suggest that levels of ortho-Toluidine, thiocyanate and ortho-Cresol significantly reduce after smokers switch to vaping product use for at least 5 days.

7.7 Biomarkers of exposure to carbon monoxide

Here we discuss studies that reported on exposure to carbon monoxide (CO), including expired air CO and carboxyhaemoglobin (COHb) (methods: table 3). Carbon monoxide is categorised as a reproductive or developmental toxicant by the FDA (80). Exposure to CO also contributes to an increased risk of myocardial infarction and sudden death from coronary heart disease (99). Both CO exposure biomarkers, expired air CO and COHb, are strongly correlated (100) and have an approximate half-life of 5 to 6 hours.

Study characteristics

The literature search identified 7 RCTs (4-8, 74, 101), 7 cross-over trials (11, 14, 15, 17, 102-104), 14 non-randomised longitudinal studies (21, 23, 105-116) and 5 cross-sectional studies (31, 37, 44, 46, 117) reporting on levels of CO biomarkers.

Of the 28 studies with more than one time-point reporting on CO exposure, 10 were conducted in the US (4-7, 15, 17, 21, 74, 115, 116), 4 in Italy (101, 106, 110, 114), 3 in the UK (8, 102, 109), 2 in Greece (107, 111), 2 in Poland (23, 105), one in Canada (11), one in Germany (108), one in Ireland (113) and one in Malaysia (103). Three RCTs (7, 8, 74) and one non-randomised longitudinal study (113) were supported by the tobacco industry, and one longitudinal study was supported by a vaping product company which later was bought by the tobacco industry (106) (appendices: table 5).

Of the 5 cross-sectional studies reporting on CO, 3 were from the US (31, 44, 46), one from Canada (117) and one from Spain (37). One cross-sectional study (44) was funded by the tobacco industry (appendices: table 5).

Sample sizes of the included studies ranged from 18 in a cross-over (116) and a longitudinal (17) study to 520 in an RCT (4). Participants' age across studies ranged from a mean of 22 to 23 years (104, 105) to 62.8 in a longitudinal study following up smokers who participated in early lung screening program (101), and between 13.3% (103) and 64% (109) of participants were women with the exception of some studies that recruited only male participants (14, 102, 112). One RCT recruited only African American and Latinx smokers (6). One non-randomised longitudinal study explored health effects of vaping product use for 12 weeks in HIV-positive participants (115), one investigated a vaping

product use intervention for 12 weeks in homeless participants accessing a temporary accommodation service in Ireland (113) and one longitudinal study explored vaping product use for 24 weeks among adults with schizophrenia spectrum disorders who smoked cigarettes (106). Most studies recruited smokers or dual users and explored changes in CO levels after vaping product use, however 2 studies differed in their methodology (112, 114). Polosa and others recruited daily vaping product users who had never smoked or smoked less than 100 tobacco cigarettes in their life and followed-up them for 42 months (114), while Barna and others also recruited healthy daily vaping product users who had been heavy smokers in the past and asked them to return to regular smoking of 20-25 tobacco cigarettes for a week (112).

Sample sizes from cross-sectional studies ranged between 88 (37) and 422 (46), with female participants making up between 23.5% (37) and 66% (31) of non-specific general population samples. One study sampled specifically among those from ‘American Indian’ descent (31). One study recruited participants who were binge drinking (117) and another investigated levels among those using different types of vaping products (44).

RCTs

A total of 1576 participants were recruited across the 7 RCTs (4-8, 74, 101). All RCTs recruited participants who smoked at least 5 (5, 6) or 10 (4, 7, 8, 74, 101) cigarettes per day. The 3 tobacco-industry-funded RCTs were conducted in confinement with follow-up periods of 5 (7, 74) or 7 days (8), and RCTs funded independently followed up participants for 6 (6), 8 (5) or 24 weeks (4, 101). The RCTs randomised participants to vaping with nicotine, vaping without nicotine, dual use, smoking, use of NRT, use of HTP or abstinence.

Cross-over studies

Seven cross-over studies (11, 14, 15, 17, 102-104) reported on exposure to CO among a total of 168 participants. One cross-over trial recruited all male former smokers who were daily vaping product users (14), 2 studies recruited dual users (11, 15), one study recruited smokers of at least one tobacco cigarette per day (102), and 3 studies recruited smokers of at least 10 cigarettes per day (17, 103, 104). Four cross-over trials explored acute exposure to vaping product use (14, 17, 102, 104) and 2 studies included 5- (15) and 7-day (11) cross-over conditions.

Longitudinal studies

Fifteen non-randomised longitudinal studies (21, 23, 103, 105-116) reported on CO changes in 622 participants. Follow-up lengths ranged from acute vaping product exposure (103, 105) to 42 months (114).

Cross-sectional studies

Definitions of vaping differed across studies. One study investigated participants who were daily vapers (31), and 2 did not report the frequency of vaping required for participation (37, 44). Two did not include vapers and only reported on dual users (46, 117) and no study investigated non-users.

Risk of bias of included studies

RCTs

All 7 RCTs were assessed to have some concerns in relation to overall risk of bias according to the RoB2 risk of bias tool (appendices: table 1). The key concerns were a lack of information on the randomisation process and lack of pre specified data analysis plans.

Cross-over studies

Of the 7 cross-over studies, 4 were assessed as having some concerns in overall risk of bias (14, 17, 102, 104) and 3 were assessed as having high concerns in overall risk of bias (11, 15, 103) according to the RoB2 risk of bias tool for cross-over trials (appendices: table 2). Two cross-over studies that included 5- and 7-day conditions were assessed at high risk of bias due to potential deviations from intended interventions (confounding of continued smoking during vaping and non-use conditions) (11, 15), and the third study was assessed at high risk of bias due to a lack of participants' randomisation (103).

Longitudinal studies

Of the 15 non-randomised longitudinal studies, 12 were assessed at moderate risk of bias (21, 23, 105, 106, 108, 109, 111-116) and 3 were assessed at serious risk of bias (103, 107, 110). Most studies at moderate risk of bias were assessed that way due to risk of confounding associated with continued smoking, except for Polosa and others, which was at risk of bias in participant selection (participants had been vaping for different lengths of time before being enrolled to the trial) and missing data (114), and Ruther and others, which was at risk of bias in participant selection, classification of interventions and missing data (108). Beatrice and others was assessed at serious risk of bias due to confounding regarding smoking (110), Ikonomidis and others was assessed at serious risk of bias because of deviations from intended interventions that have been assessed at follow-up (107) and Nga and others was assessed at serious risk of bias because selection into the study was related to intervention (103).

Cross-sectional studies

Quality of all cross-sectional studies was assessed using Biocross quality appraisal tool and is reported in the appendices (table 4). Studies reporting levels of CO scored between

4 (37) and 16 (44) out of a maximum score of 20, with most studies of reasonably good quality. The main limitations were associated with lack definitions of vaping, lack of detail about statistical adjustments for confounders and limited detail on laboratory measurement procedures (for example, blinded analyses, reporting on quality control procedures).

Study findings

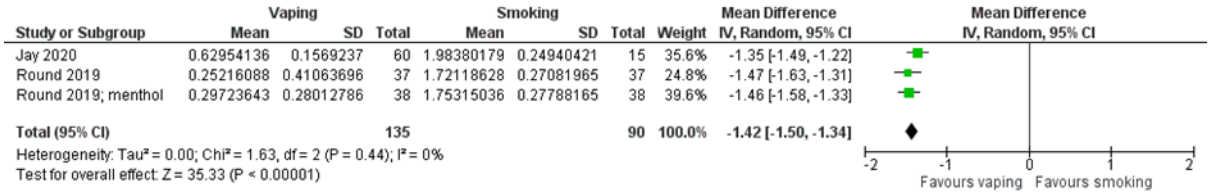
RCTs

Four RCTs (4, 6, 8, 101) reported on changes in expired air CO after switching from smoking to vaping but only McEwan and others (8) measured changes after a complete switch from smoking to vaping product use (table 36). Seven days after smokers switched to vaping product use in confinement, their expired air CO levels had statistically significantly decreased by 84.4% compared with baseline, which was similar to significant CO reductions within participants assigned to non-use (85.7%) and HTP use (82.8%) groups and significantly lower than in smokers (an increase by 4.1%).

Average changes in CO exposure in the other 3 RCTs were likely dependent on how many participants adhered to the vaping only condition. At 6 weeks follow up in Pulvers and others trial (6), 28.1% of participants in the vapers group had completely switched to vaping product use and another 57.9% were dual users, which resulted in a statistically significant reduction of 56.3% in expired-air CO (6). At 24 weeks follow up in the Cobb and others trial (4), all participants within vaping product use subgroups (randomised to using 0mg/mL, 8mg/mL and 36mg/mL nicotine e-liquid) continued smoking on average 6 to 8 cigarettes per day, and reductions in expired-air CO levels ranged from statistically not significant 14.6% in the 0mg/mL condition, to a statistically significant 18.1% reduction in the 8mg/mL and 26.7% reduction in the 36mg/mL conditions (4). Lucchiari and others (101) also reported that a small proportion of participants randomised to vaping product use completely switched to exclusive vaping at 6 months follow up (19% and 16% in nicotine and non-nicotine vaping groups respectively), therefore changes in expired-air CO levels were not statistically significant within groups (101).

Three RCTs reported on the change in carboxyhaemoglobin (COHb) levels after switching from smoking at least 10 cigarettes per day to vaping product use in confinement for 5 days (7, 74) or from smoking at least 5 cigarettes per day to ad libitum vaping product use for 8 weeks (5). We meta-analysed data on change in COHb levels between vapers and smokers from 2 tobacco industry funded RCTs (figure 53).

Figure 53. Meta-analysis of RCTs reporting on blood COHb levels (carbon monoxide) after exposure to vaping and smoking

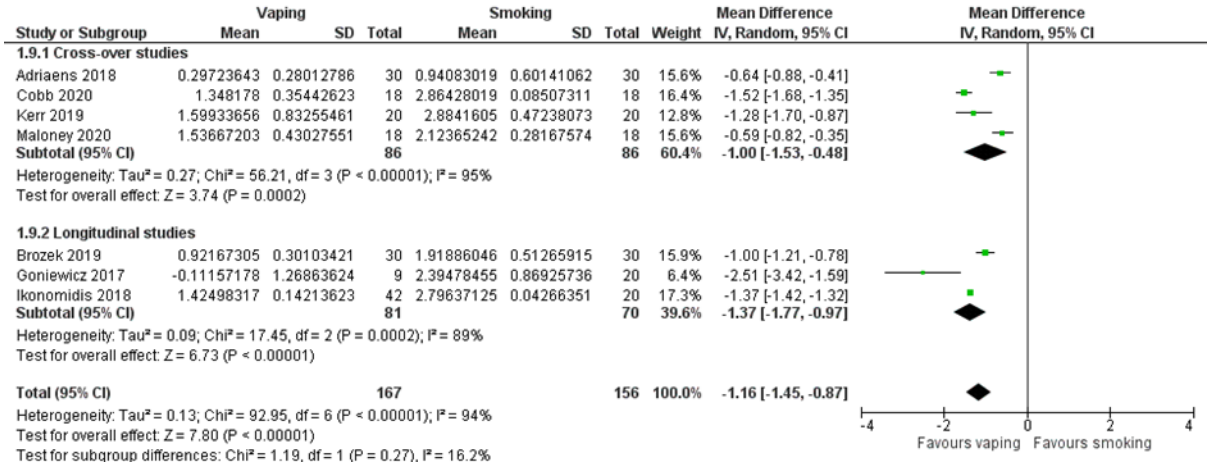


The average blood COHb levels were statistically significantly lower among vapers than smokers in 2 RCTs (LMD: -1.42, 95% CI: -1.50, -1.34; 225 participants); the geometric mean COHb levels were approximately 76% lower among vapers than among smokers (GMR: 0.24, 95% CI: 0.22, 0.26). There was no heterogeneity between the studies at I^2 = 0%. An RCT by Hatsukami and others reported statistically significant average reductions in blood COHb levels in vaping product users (58%), nicotine gum or lozenge users (45%) and dual users (22%) 8 weeks after switching from smoking (5). The average reduction was statistically significantly higher among exclusive vaping product users than dual users (5), in line with CO being associated with combustible tobacco use.

Cross-over studies

Six cross-over studies reported on change in expired air CO levels after exposure to vaping product use (11, 14, 15, 17, 102, 104) and none reported on COHb levels (table 36). Four of the studies (14, 17, 102, 104) explored acute vaping product use effects on expired air CO change and 2 studies included vaping product use conditions for 5 (15) and 7 (11) days. We meta-analysed data on the change in expired air CO levels between vapers and smokers from 4 cross-over and 3 non-randomised longitudinal studies that met the meta-analysis inclusion criteria (methods: table 6, figure 54). The average expired air CO levels were statistically significantly lower among vapers than smokers' groups in 4 cross-over trials (LMD: -1.00, 95% CI: -1.53, -0.58; 172 participants); the geometric mean CO levels were approximately 63% lower among vapers than among smokers (GMR: 0.37, 95% CI: 0.22, 0.56). There was considerable heterogeneity between the studies at I^2 = 95%, but the direction of the difference was consistent across reported findings. Another cross-over trial reported that expired air CO levels did not differ between vapers after 10 puffs of a modular type vaping product with 1.5mg/mL nicotine e-liquid, after 10 puffs of the same vaping product with nicotine-free e-liquid and after sham vaping (14). The Czoli and others study (11), which included 7-day long ad libitum vaping, smoking and no use cross over conditions, reported statistically significant reductions in expired CO levels during vaping (reduction of ~40.8%) and non-use (reduction of ~26.0%) conditions (11). However, adherence to study conditions was not enforced, and authors reported that participants on average smoked 1.9 cigarettes per day during vaping condition and 3 cigarettes per day during non-use condition (11).

Figure 54. Meta-analysis of cross-over and longitudinal studies reporting on expired air CO levels after exposure to vaping and smoking



Longitudinal studies

Of the 15 non-randomised longitudinal studies, all reported on expired air CO levels (21, 23, 103, 105-116) and 2 reported on COHb changes (110, 112) (table 36).

Data from 3 longitudinal studies that reported on participants' expired air CO levels after acute vaping product exposure (105) or followed-up smokers who switched to ad libitum vaping product use for 2 weeks (23) or a month (107) were pooled and meta-analysed (figure 54). The average expired air CO levels were statistically significantly lower among vapers than smokers' groups in 4 cross-over trials (LMD: -1.37, 95% CI: -1.77, -0.97; 151 participants); the geometric mean CO levels were approximately 63% lower among vapers than among smokers (GMR: 0.25, 95% CI: 0.17, 0.38). There was considerable heterogeneity between the studies at I^2 = 89%, but the direction of the difference was consistent across reported findings. Reduction in expired air CO levels was reported in all included longitudinal studies where smokers switched to vaping product use for 2 weeks (109), 4 weeks (21), 10 weeks (116), 3 (108), 4 (111) and 6 months (110). The CO reduction was also reported in studies where participants assigned to vaping product use continued to smoke and it was associated with a reduced number of tobacco cigarettes smoked per day (106, 113, 115).

A higher exposure to CO in smokers than vaping product users was demonstrated in Barna and others (112) where daily vaping product users (who used to be heavy smokers) were asked to switch to smoking 20-25 cigarettes per day for a week—authors reported statistically significant over 6-fold increase in expired air CO levels after a week of smoking. Polosa and others (114) followed up daily ad libitum vaping product users who have never smoked for 42 months and reported median CO levels were similar between vaping product users and non-users' groups.

The Barna and others study reported a statistically significant 2-fold increase in blood COHb levels of daily vaping product users after a week of smoking over 20 cigarettes per day (112) and Beatrice and others reported a statistically significant reduction in blood COHb levels by over 80% 6 months after switching from smoking to a disposable vaping product use with 18mg/mL nicotine e-liquid (110).

Table 36. Randomised controlled trials, cross-over and longitudinal studies reporting on levels of carbon monoxide biomarkers among vapers

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
CO							
Breath biosample							
RCT							
Cobb et al., 2021, US (4)	24 weeks (S-M)	See: Cobb et al., 2021	8 mg/mL, n=74 18.78 (16.53-21.02) ppm (Br) ↓**18.1%** 36 mg/mL, n=80 16.72 (14.64-18.72) ↓**26.7%** 0 mg/mL, n=69 19.62 (17.33-21.92)	NA	NA	n=91 20.5 (18.56-22.43) ↓13.5%	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			↓14.6%				
Lucchiari et al., 2020, Italy (101)	6 months (S-M)	n = 210 Smokers: participants of an early lung cancer screening program, ≥55 years old, smoked ≥10 TC per day for a last 10 years. Mean (SD): 62.8 (4.6), 37.1% females. Vaping (n=70): ad lib use of a VP with 8 mg/mL nicotine strength vaping liquid and low-intensity stop smoking counselling. Non-nicotine vaping (n=70): ad lib use of the same VP with nicotine-free vaping liquid and low-intensity stop smoking counselling. Smoking (n=70): ad lib use of own-brand TC with low-intensity stop smoking counselling. Adherence at 6 month FU: Vaping group: 19% (n=13) stopped smoking. Non-nicotine vaping group: 16% (n=11) stopped smoking. Smoking group: 10% (n=7)	a1) Vaping, n=52 12.01 (8.1)[a2,c] ppm (Br) ↓21.7% a2) Non-nicotine vaping, n=51 15.28 (11.4)[a1,c] ppm ↑4.8%	NA	n=52 16.52 (10.2)[a1,a2] ↑12.9%	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		stopped smoking.					
McEwan et al., 2021, UK (8)	7 days (A)	See: McEwan et al., 2021	n=28 4.1[c] ppm (Br) ↓**84.4%**	NA	n=30 25.3[a,d,e] ↑4.1%	n=29 3.2 [c] ↓**85.7%**	n=28 4.4 [c] ↓**82.8%**
Pulvers et al., 2020, US (6)	6 weeks (S-M)	See: Pulvers et al., 2020	n=114 Median (IQR)=7 (3; 14)[c] ppm (Br) ↓**56.3%**	NA	n=54 16 (9; 25)[a] ↓5.9%	NA	NA
Cross-over							
Adriaens et al., 2018, Belgium (104)	Single use (A)	n = 30 Smokers: TC smokers for ≥3 years, smoking ≥10 TC per day, having no intention to stop smoking. Mean (SD) age: 22 (3.1), 67% males. Vapers (n=30): 5 minutes of ad lib use of tank VP (Eleaf iStick Power with 5000 mAh battery at 8 W, Aspire Nautilus 2 tank containing a 1.6 Ω coil) with 70/30 PG/VG ratio liquid with 18 mg/ml nicotine of either tobacco or menthol flavour.	n=30 20 min: 3.17 (0.34)[c] ppm (Br) ↑12% 50 min: 2.83 (0.3)[c] ppm (Br) 0%	NA	n=30 20 min: 7.67 (0.49)[a,d] ↑**147.4%** 50 min: 6.47 (0.41)[a,d] ↑**108.7%**	NA	n=30 20 min: 3.57 (0.38)[c] ↑**30.8%** 50 min: 3.07 (0.32)[c] ↑**12.5%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		Smokers (n=30): 5 minutes of ad lib use of own-brand TC. Other (n=30): 5 minutes of ad lib use of HTP (IQOS).					
Chaumont et al., 2020, Belgium (14)	Single use (A)	See: Chaumont et al., 2020	Nicotine: 4 (3-5) ppm (Br) Non-nicotine: 4.5 (3-5.8) ppm (Br)	NA	NA	NA	3.5 (3-5)
Cobb et al., 2020, US (15)	5 days (A)	See: Cobb et al., 2020	n=22 EEM (SEM)=4.1 (1.5)[b,c] ppm (Br) ↓75.4%	n=22 16.5 (1.5)[a,d] ↓8.8	n=22 17.6 (1.5) [a,d] ↓7.2%	n=11 Mean (SEM)=1.9 (0.5) [b,c] ↓83.5%	NA
Czoli et al., 2019, Canada (11)	7 days (A)	See: Czoli et al., 2019	n=48 10.33 (7.47-13.18) ppm (Br) ↓**40.8%**	NA	n=48 21.12 (17.4-24.9) ↑**21.0%**	n=48 12.91 (10.2-15.6) ↓**26.0%**	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Kerr et al., 2019, UK (102)	Single use (A)	n = 20 Smokers: smoking ≥1 TC per day. Mean (SD) age: 31.6 (10.5), 100% males, mean (SD) BMI: 25.7 (5). Vaping (n=20): 15 puffs on a tank type VP (1300mAh, 3.3 V battery) with 66%/34% PG/VG ratio, 18 mg/mL nicotine strength and tobacco flavoured vaping liquid. Smoking (n=20): ad lib smoking of a TC.	n=20 7 (2)[c] ppm (Br) ↓**22.2%**	NA	n=20 10 (2)[a] ↑**122.2%**	NA	NA
Maloney et al., 2020, US (17)	Single use (A)	See: Maloney et al., 2020	Monitored use: 5.1 (2.3)[c] ppm (Br) ↓7.3% Ad lib use: 4.7 (1.9) [c] ppm (Br) ↓11.3%	NA	Monitored use: 8.7 (2.5)[a,e] ↑**67.3** Ad lib use: 16.6 (7) [a,e] ↑**88.6%**	NA	Monitored use: 4.9 (1.8) [c] ↓7.5% Ad lib use: 4.3 (1.4) [c] ↓6.5%

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Longitudinal							
Barna et al., 2019, Hungary (112)	7 days (A)	n = 24 Vapers: past heavy smokers who used a VP with ≥10 mg/mL nicotine at baseline. Age range: 20-64, 100% males. Smoking (n=24): vapers switched to smoking 20-25 own-brand TC a day for 7 days.	NA	NA	Median = 15 ppm (Br) ↑**650%**	NA	NA
Beatrice et al., 2019, Italy (110)	6 months (S-M)	n = 40 Smokers: on average smoking 21.7 TC per day for 31 years, unwilling or unable to stop smoking, willing to switch to an alternative nicotine product. Mean age: 49.8, 100% males. Vapers (n=20): using disposable VP with 18 mg/ml nicotine. Other (n=20): using HTP (IQOS) with mean nicotine of 0.5 mg per stick.	n=20 Median (IQR): 2 (1; 2,75) ppm (Br) ↓**83.3%**				n=20 3 (3;4) ↓**72.7%**
Brozek et al., 2019, Poland (105)	Single use (A)	n = 120 Smokers (n=30): self-reported smoking status. Mean (SD) age: 23.2 (1.6), 50% females. Dual users (n=30): self-reported smoking and VP use status. Mean (SD) age: 22.3 (2.7),	n=30 2.63 (0.81) ppm (Br) ↑8.2%	n=30 5.63 (3.79) ↓2.4%	n=30 7.77 (4.26) ↑**89.5%**	After 1 minute, n=30 2.03 (0.67) ↑6.8%	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		26.7% females. Vapers (n=30): self-reported VP use status. Mean (SD) age: 22.2 (2.3), 36.7% females. Non-users (n=30): self-reported non-smoking status. Mean (SD) age: 22.9 (1.9), 50% females. Vaping (n=30): vapers ad lib used their own-brand VP with 12 mg/ml nicotine and multi-fruit flavoured vaping liquid for 5 minutes. Dual use (n=30): dual users ad lib used their own-brand VP with 12 mg/ml nicotine and multi-fruit flavoured vaping liquid for 5 minutes. Smoking (n=30): smokers ad lib smoked a TC (0.6 mg nicotine per TC). Non-use (n=30): non-users simulated use of a VP.					
Caponnetto et al., 2021, Italy (106)	24 weeks (S-M)	n = 40 Smokers: adult outpatients at psychiatric clinics, smoking >19 CPD, not intending to reduce or stop smoking, having a schizophrenia spectrum disorder diagnosis without a recent	n=37 Week 12 8.2 (6.5) ppm ↓75.9%	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		exacerbation. Mean (SD) age: 48.3 (12.1), 35% females, 100% white Caucasian, mean (SD) age onset of schizophrenia spectrum disorders: 21.9 (2.8). Vaping (n=40): ad lib use of pod VP (JUUL, 200 mAh integrated battery) with Virginia tobacco flavour 5% nicotine salt pods. Participants informed to use the VP as much as they like with free pods for 12 weeks. Adherence: Mean (SD) CPD: baseline—28 (9.1), week 12—6.4 (6.9), week 24—6.9 (6.8).	Week 24 9.3 (8.6) ↓72.6%				
Cioe et al., 2020, US (115)	12 weeks (S-M)	n = 20 Smokers: HIV-positive and in-care for the condition, not wanting to stop smoking in the next 30 days. Smoked ≥5 TC per day for >1 year. Mean (SD) age: 52.7 (9.3), 30% females, 50% non-Hispanic white, 25% non-Hispanic Black or African American, 15% Hispanic, 10% non-Hispanic Multi-Racial, mean (SD) years	n=19 6.74 (3.89) ppm (Br) ↓**57.1%**	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		living with HIV: 21.1 (10.2). Vaping (n=20): ad lib use of cartridge VP (ce6 eGo-T 3.3 V, 1100 mAh batteries with 6-10 1.5 Ω, dual coil XL, 510-style Smoktech cartomizers) with 30/70 PG/VG ratio liquid with 18 mg/mL nicotine and a choice of tobacco, menthol or fruit flavours. Adherence: Mean (SD) of CPD at baseline: 15.1 (9.6), at 12-week follow-up: 2.44 (4.01). 7 participants self-reported switching completely to VP.					
Goniewicz et al., 2017, Poland (23)	2 weeks (S-M)	See: Goniewicz et al., 2017	n=9 Mean=2 ppm (Br) ↓**81.8%**	n=11 6 ↓68.4 %	NA	NA	NA
Ikonomidis et al., 2018, Greece (107)	Single use (A) and 1 month (S-M)	n = 70 Smokers: people attending hospital's smoking cessation unit. Mean (SD) age: 48 (5), 56% females.	1) Nicotine acute, n=35 12.1 (0.5) ppm (Br) ↓6.9%	1 month, n=24 12.5 (0.6)	1) Acute, n=35 14.2 (0.8) ↑10.1%	Acute, n=70 12.7 (0.9) ↓1.6%	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		Vaping, acute (n=35): 7 minutes use of a cartridge VP (NOBACCO eGo Epsilon, 1100 mAh, 3.9 V) with 74.3%/20% PG/VG ratio, 0 mg/mL or 12 mg/mL nicotine strength vaping liquid. Smoking, acute (n=35): smoking a TC for 7 minutes. Non-use, acute (n=70): sham smoking for 7 minutes on a nonlighted TC. Vaping, 1 month (n=42): ad lib use of the same VP. Dual use, 1 month (n=24): ad lib use of the VP and own-brand TC. Smoking, 1 month (n=20): ad lib smoking of own-brand TC.	2) Non-nicotine acute, n=35 12.0 (0.6) ↓7.0% 3) Vaping, 1 month, n=42 4.2 (0.6) ↓**69.8%**	↓**16.7 %**	2) 1 month, n=20 16.4 (0.7) ↑7.9%		
Ikonomidis et al., 2020, Greece (111)	4 months (S-M)	n = 40 Smokers: without cardiovascular diseases. Vaping (n=20): ad lib use of a cartridge VP (NOBACCO eGo Epsilon, 1100 mAh, 3.9 V) with 74.3%/20% PG/VG ratio, 12 mg/mL nicotine strength vaping liquid. Adherence confirmed by <10ppm and self-reported	n=20 5.6 (3.8) ppm (Br) ↓**55.2%**	NA	n=20 10.2 (3.8) ↓20.3%	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		abstinence from smoking at follow-up. Mean (SD) age: 46.8 (10.9), 75% females. Smoking (n=20): ad lib smoking of own-brand TC. Mean (SD) age: 43.2 (11.7), 85% females.					
Kimber et al., 2021, UK (109)	2 weeks (S-M)	n = 50 Smokers: smoking ≥5 TC per day, have been smoking for >1 year, not using a VP. Mean (SD) age: 29.5 (9.3), 64% females, 60% white, 10% black, 6% mixed and 4% of Asian ethnicity. 1) Vaping, cartridge VP (n=11): ad lib use of a cartridge type VP (TECC Go and Blu) with 18 mg/mL strength nicotine and 50%/50% PG/VG ratio vaping liquid. Adherence: mean CPD at week 2=5.4. 2) Vaping, tank type VP, 18 mg/ml (n=20): ad lib use of a tank type VP (Totally Wicked mini curve, 1.5 Ω resistance) with 18 mg/mL strength nicotine	1) Cartridge VP, n=8 9.91 (4.88-14.94) ppm (Br) ↓**25.8%** 2) Tank VP 18 mg/mL, n=20 10.55 (6.8-14.3) ↓**36.4%** 3) Tank VP 6 mg/mL, n=19 12.21 (8.4-16.0)	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		and 50%/50% PG/VG ratio vaping liquid. Adherence: mean CPD at week 2=4.6. 3) Vaping, tank type VP, 6 mg/mL (n=19): ad lib use of the same tank type VP with 6 mg/mL strength nicotine and 50%/50% PG/VG ratio vaping liquid. Adherence: mean CPD at week 2=6.9.	↓**23.7%**				
Nga et al., 2020, Malaysia (103)	Single use (A)	n = 45 Smokers: smoking ≥10 TC per day for ≥5 years with CO levels of ≥10 ppm at screening. Mean age: 43.6 years, 13.3% females, 51.1% of Chinese ethnicity. Vapers (n=15): 10 puffs with 30 s inter-puff intervals of a tank VP (Aspire AVP AIO Kit 700 mAh battery with a 1.2 Ω coil) with 70/30 PG/VG ratio liquid with 10 mg/mL nicotine of tobacco flavour. Smokers (n=15): smoking of own brand TC. Other (n=15): single use of HTP	n=15 15 min: 8.8 (1.56)[c,e] ppm (Br) ↑**113.0%** 45 min: 6.4[c,e] ppm (Br) ↑**55.0%**	NA	n=15 15 min: 17.2[a,d] ↑**316.5%** 45 min: 16.47 [a,d] ↑**298.8%**		n=15 15 min: 5.47[a,c] ↑**32.4%** 45 min: 4.67 [a,c] ↑**13.1%**

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		(IQOS).					
Polosa et al., 2017, Italy (114)	42 months (L)	n = 21 Vapers: never smoked or smoked <100 TC in their lifetime, daily use of a VP for ≥3 months. Non-users: age- and sex-matched non-users of tobacco or nicotine products selected as a control group. Vaping (n=16): ad lib use of own-brand tank type VP with 0% (3/9), 0.9% (2/9), 1.2% (2/9), 1.6% (1/9) and 1.8% (1/9) nicotine strength vaping liquid with tobacco (7/9), mint (1/9) or fruit (1/9) flavours. Non-use (n=15): never smokers or vapers.	n=9 Median (IQR)=4 (2.8; 6.3) ppm (Br) ↓20%	NA	NA	n=12 5 (5.5; 6.0) ↑25%	NA
Pulvers et al., 2018, US (21)	4 weeks (S-M)	See: Pulvers et al., 2018	NR	n=40 8.93 (8.35) ppm (Br) ↓37.5 %	NA	NA	NR

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Rohsenow et al., 2018), US (116)	10 weeks (S-M)	n = 18 Smokers: smoking >10 TC per day for past 6 months, CO > 8 ppm at baseline. Mean (SD) age: 45.1 (7.8), 61% females, 94% white, 6% Black, 11% Hispanic. Vapers (n=18): everyday ad lib use (at least taking 5 puffs per day) of a modular VP (Smoktech dual coil, 1.5 Ω, size XL cartomizers and eGo battery, 3.3 V, 1100 mAh) with 18 mg/mL nicotine liquid of tobacco, menthol, chocolate dessert or mixed fruit flavours.	n=17 11.25 (6.38) ppm (Br) ↓35.1%	NA	NA	NA	NA
Ruther et al., 2021, Germany (108)	3 months (S-M)	n = 54 Smokers: smoking ≥5 years, ≥10 TC per day wishing to switch to VP or stop smoking. 1) Vaping (n=60): switch from smoking to ad lib use of own-brand VP. Mean (SD) age: 39.1 (12.8), 26.7% females, mean (SD) BMI: 25.3 (30). 2) Non-use (n=20): stopping smoking with a controlled	n=40 9.7 (5.7) ppm (Br) ↓**24.2%**	NA	NA	n=14 6.9 (8.0) ↓36.1%	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
		smoking cessation program. Mean (SD) age: 44.2 (11.7), 50% females, mean (SD) BMI: 23.9 (3.3). Adherence: In vapers group at 3-month FU (n=40), 11 were sole VP users and 29 were dual users. In non-users' group at FU (n=14), 9 stopped smoking and 5 continues smoking.					
Scheibein et al., 2020, Ireland (113)	12 weeks (S-M)	n = 23 Smokers: homeless, users of supported temporary accommodation services, wish to give up smoking, >5 ppm of exhaled breath CO. Among those followed-up (n=9), mean (SD) age: 43.9 (7.4), 22.2% females, mean (SD) CPD: 25.2 (7.8). Vaping (n=9): ad lib use of a tank type VP (Endura T22e) with a variety of nicotine strength vaping liquids (0 to 20 mg/mL) and with tobacco (n=7) or berry (n=2) flavours. Adherence: CPD decreased from 26.7 to 9 a day at 12-weeks follow up.	n=9 Mean = 16.1 ppm (Br) ↓26.5%	NA	NA	NA	NA

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
Carboxyhaemoglobin (COHb)							
Blood biosample							
RCT							
Hatsukami et al., 2020, US (5)	8 weeks (S-M)	See: Hatsukami et al., 2020	n=58 Geometric means ratio (95% CI) = 0.42 (0.33-0.55)[b] (BP) ↓**58%**	n=64 0.78 (0.65-0.93)[a] ↓**22%**	n=32 0.85 (0.69-1.06) ↓15%	NA	n=52 0.55 (0.43-0.69) ↓**45%**
Jay et al., 2020, US (74)	5 days (A)	See: Jay et al., 2020	n=60 1.9 (0.3) % (BP) ↓**72.9%**	NA	n=15 7.5 (1.9) ↑11.9%	n=11 1.9 (0.4) ↓**72.1%**	NA
Round et al., 2019, US (7)	5 days (A)	See: Round et al., 2019	Non-menthol, n=37: 1.4 (0.6) % saturation (B) ↓**75.9%** Menthol, n=38: 1.4 (0.4) % saturation	NA	NA	NA	Non-menthol, n=38: 1.3 (0.4) ↓**75.9%** Menthol, n=40: 1.4 (0.5)

Author, year, country	Last follow-up (exposure length[1])	Baseline participants' characteristics and grouping/assignment[2]	Level of a biomarker at the last follow-up (biosample type[3]), % change from baseline*				
			Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]	Other[e]**
			(B) ↓**76.7%**				↓**75.4%**
Longitudinal							
Barna et al., 2019, Hungary (112)	7 days (A)	See: Barna et al., 2019	NA	NA	Median = 3.05 % (BP) ↑**205%**	NA	NA
Beatrice et al., 2019, Italy (110)	6 months (S-M)	See: Beatrice et al., 2019	n=20 Median (IQR): 0.32 (0.16; 0.44) (Bl) ↓**83.3%**	NA	NA	NA	n=20 0.48 (0.48; 0.64) ↓**72.7%**

Notes: * Within-group changes are calculated as ((Baseline level – Follow-up level)/Baseline level) * 100.

** Other group refers to an additional comparison group to vapers, dual users, smokers and non-users that is defined in participants' grouping/assignment column.

[1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Grouping refers to randomisation groups (for RCT studies), cross-over conditions (cross-over studies) or follow-up groups (longitudinal studies).

[3] Biosample types: Br—breath, Bl—blood, BP—blood plasma, S—saliva, U—urine.

Level of change in bold represents significant within group change from baseline, $p < 0.05$.

[a,b,c,d,e] RCT and cross-over conditions with different superscript letters were statistically significantly different from one another, $p < 0.05$.

Cross-sectional studies

Two studies compared expired air CO levels between smokers and vapers, reporting levels to be on average between 47% (37) and 75% (31) lower among vapers compared to smokers, both differences were statistically significant (table 37). Gonzalez-Roz and others did not provide detail on how they defined vaping and reported a mean CO level of 8 ppm among vapers, suggesting that some current smokers were included in the vapers sample as this is higher than would be expected from a non-smoker (37).

Table 37. Cross-sectional studies reporting on levels of carbon monoxide biomarkers among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
Carbon monoxide (CO)					
Breath biosample					
Carroll et al., 2018, US (31)	See: Carroll et al., 2018	**3.05 (2.3-4.04)**[b,c] **ppm (Br)**	**14.6 (10.9-19.6)**[a] **0.209**	**12.18 (9.27-13)**[a] **0.250**	NA
González-Roz et al., 2017, Spain (37)	See: González-Roz et al., 2017	**8 (6.77)**[c] **ppm (Br)**	NA	**15.24 (7.18)**[a] **0.525**	NA
González-Roz et al., 2021, Canada (117)	n1 = 339 Mean (SD) age: 36.5 (12.4), 50.4% females, 82.6% white, 1.5% Black/African, 9.4% Asian, 2.4% of other ethnicity. n2 = 174, 19-23 years old people who were binge drinkers Mean (SD) age: 21.4 (1.2). 50.5% females, 77.1% white, 2.0% Black/African, 14.7% Asian, 1.7% of other ethnicity Sample 1: Dual users (n=72): VP and TC use at least once during past 30 days. Smokers (n=242): TC use at least once during past 30 days.	NR	**Sample 1: 13.46 (15.45) ppm (Br)** **Sample 2: 7.26 (8.09) ppm (Br)**	**Sample 1: 14.4 (13.75)** **0.935** **Sample 2: 7.12 (7.26)** **1.020**	NA

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smoking[c]	Non-users[d]
	Sample 2: Dual users (n=76): as for Sample 1. Smokers (n=174): as for Sample 1.				
Piper et al., 2019, US (46)	See: Piper et al., 2019	NA	**16.29 (11.02) ppm (Br)**	**16.73 (9.64)** **1.03**	NA
Carboxyhaemoglobin (COHb)					
Blood biosample					
Oliveri et al., 2020, US (44)	See: Oliveri et al., 2020	Tank VP: 2.8 (2.2) % saturation (B) Cartridge VP: 3 (2.2) % saturation (B)	4.9 (2.6) 0.571 (vs tank VP) 0.612 (vs cartridge VP)	NA	NA

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Summary of studies reporting on exposure to carbon monoxide

In general, data from the included longitudinal studies suggest that exposure to carbon monoxide can be substantially reduced after completely switching from smoking to vaping product use. Among dual users, the degree of CO exposure reduction is dependent on the amount of tobacco cigarettes that are smoked. Some interventional studies suggest that exposure to CO in smokers who completely switch to vaping product use might be reduced to the levels similar to non-users.

In this review, there was limited cross-sectional research investigating levels of carbon monoxide among vapers. Evidence indicates that levels of carbon monoxide are substantially lower among vapers compared to smokers. However, limited control over vapers' current and historical smoking behaviour are likely to have affected the levels of carbon monoxide measured.

7.8 Biomarkers of exposure to metals

Tobacco plants absorb metals from the soil and fertilisers, and tobacco combustion liberates the metals which are retained in ash or transferred to tobacco smoke. Smokers have high levels of some metals in their blood that, because of their long half-lives (for example, years in the case of cadmium), can be detectable in human biosamples for many years after past tobacco exposure or exposure to other environmental sources of metals.

Metals have also been found in the vaping product aerosols. Vaping products can contain a range of different types of metal elements depending on the type of device. Exposure to metals and metalloids may originate from the atomiser and from soldered joints and other parts of the device such as batteries which may leach into the e-liquid (3). As the design of vaping products have evolved over time, so have the metal components, particularly those in atomisers and batteries. Early vaping products such as cig-a-likes contained a filament, thick wires, wire joints, sheath and fibres while later models lack some of these components (118). Some metal compounds may be present in vaping products but not in tobacco smoke. Exposure to certain metals can be carcinogenic and can have effects on cardiovascular and respiratory systems (methods: table 3).

We did not identify any longitudinal studies but found 10 cross-sectional studies that reported on differences in exposure to metals between vaping product users, smokers and non-users (table 38).

Study characteristics

Ten cross-sectional studies reported on levels of metals in urine or blood samples of vapers, smokers or non-users. Two reported on arsenic (36, 119), all 10 reported on

cadmium (36, 47, 54, 55, 65, 67, 119-122), 9 reported on lead (36, 47, 54, 55, 67, 119-122) and 2 on mercury (67, 119).

Six studies were from the US (36, 47, 54, 55, 67, 120), 2 from Poland (121, 122), one from Romania (119) and one from South Korea (65).

Samples sizes ranged from 88 (121) to 5,101 (36). One study focused specifically on women (47), and one study compared pregnant women to non-pregnant women (54). Between 51% and 76.5% of the general population participants were women. One study stratified a general population sample by those with and without respiratory symptoms (55).

Of the 10 studies, 4 used data from Wave 1 of the PATH study (36, 47, 54, 55), 2 used data from the NHANES study (67, 120), and one used data from the KNHANES study (65).

The definition of vaping varied between studies. Four investigated vapers who vaped daily or some days (36, 47, 54), one investigated vapers who vaped at least weekly (67), one investigated those who vaped at least monthly (65), one investigated those who had ever vaped (120) and 3 did not define the frequency of vaping required to participate in the study (119, 121, 122).

Risk of bias assessment

Quality of all cross-sectional studies was assessed using Biocross quality appraisal tool and is reported in the appendices (table 4). Studies reporting levels of metals scored between 12 (119) and 15 (55) out of a maximum score of 20, with most studies of reasonably good quality. The main limitations were associated with lack of detail about statistical adjustments for confounders and limited detail on laboratory measurement procedures (for example, blinded analyses or reporting on quality control procedures).

Study findings

Arsenic

Arsenic is categorised as carcinogenic compound to humans according to the IARC (79) and as a carcinogen, respiratory toxicant, reproductive or developmental toxicant in humans by the FDA (80). It has a half-life of approximately 10 hours (methods: table 3).

Two papers reported on levels of inorganic arsenic (table 38). Goniewicz and others (36) reported that urinary levels of arsenic among vapers were statistically significantly higher, by approximately 10%, compared to smokers. Badea and others (119) reported on average 60% higher levels of arsenic in blood plasma among vapers compared to

smokers, however there was wide variation between participants and the difference was not statistically significant.

When vapers were compared to non-users, Goniewicz and others reported that urinary levels of arsenic were around 2% lower among vapers than among non-users, which was not statistically different (36). Badea and others reported a 100% higher level of blood serum arsenic among vapers compared to non-users, however there was wide variation between participants and the difference was not statistically significant (119).

One study (36) reported that vapers' urinary arsenic levels were approximately 90% and non-users' levels were approximately 73% of arsenic levels detected among smokers (figure 55).

Table 38. Cross-sectional studies reporting on levels of inorganic arsenic among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Urine biosample					
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**0.053 (0.048-0.058)[c] µg/g creatinine**	**0.047 (0.045-0.05)** **1.128**	**0.048 (0.046-0.05)[a]** **1.104**	**0.054 (0.05-0.057)** **0.981**
Blood biosample					
Badea et al., 2018, Romania (119)	n = 150 Vapers' mean (SD) age: 35.2 (9.4), 76.5% females; smokers' mean (SD) age: 28.4 (10.8), 70.7% females; non-users' mean (SD) age: 24.5 (6.7), 82.8% females. Vapers (n=34): NR. Smokers (n=58): NR. Non-users (n=58) NR.	**Median (IQR): 0.16 (0.1; 0.3) ng/mL**	NA	**0.1 (0.1; 0.2)** **1.6**	**0.08 (0; 0.2)** **2.0**

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Cadmium

Cadmium is categorised as carcinogenic compound to humans according to the IARC (79) and as a carcinogen, respiratory toxicant, reproductive or developmental toxicant in humans by the FDA (80) (methods: table 3). Cadmium is a nonspecific biomarker of tobacco exposure, as it may also reflect dietary and occupational exposure (89). It has a half-life of approximately 13 years (36).

Five studies compared levels of urinary cadmium among vapers and smokers (36, 47, 54, 120, 121) (table 39). One reported statistically significantly lower levels of cadmium among vapers compared to smokers, by approximately 30% (36). One reported 52% lower levels among vapers, however this was not statistically significant (120). Coleman and others (54) reported levels to be 12% lower among non-pregnant women who vape and 23% lower among pregnant women who vape compared to those who smoked; however, differences were not statistically tested. Perez and others (47) reported no difference between female vapers and smokers. Prokopowicz and others (121) reported approximately 4% differences of cadmium among vapers compared to smokers, these differences were not statistically significant.

Four studies reported levels of cadmium in the blood among vapers and smokers (65, 67, 119, 122). Two papers reported levels of blood cadmium among vapers to be statistically significantly lower among vapers compared to smokers, ranging between 62% (67) to 69% (122). Badea and others (119) and Lee and others (65) reported levels among vapers to be between 25% and 37% lower than smokers respectively, however the difference was not statistically significant or was not tested for statistical significance.

Five studies compared levels of urinary cadmium among vapers and non-users (36, 47, 120, 121). One study reported levels to be statistically significantly higher among vapers, by around 29%, when compared to non-users (36). Three studies reported differences ranging from 17% lower to 100% higher among vapers compared to non-users, however none of these differences were statistically significant (47, 120, 121). Dai and others found that vapers with self-reported respiratory symptoms had 50% higher levels of urinary cadmium than non-users with symptoms, but the study did not test for statistical significance (55). In comparison, vapers and non-users without self-reported respiratory symptoms, had similar urinary cadmium levels, but again did not test for statistical significance (55).

Four studies also reported levels of cadmium in the blood among vapers and non-users (65, 67, 119, 122). When vapers were compared to non-users, there was little consistency in findings. Statistically significantly higher levels among vapers, of approximately 42%, were reported by Prokopowicz and others (122). Badea and others reported 25% lower levels among vapers compared to non-users, however this was not statistically significant (119). Studies using substantially larger sample sizes reported levels to be between 11%

lower and 30% higher among vapers compared to non-users, however these studies did not test for statistical significance (65, 67).

Across cross-sectional studies that measured urinary cadmium, levels among vapers were approximately between 48% and 104% and levels among non-users were between approximately 52% and 125% of cadmium levels detected among smokers (figure 55).

Table 39. Cross-sectional studies reporting on levels of cadmium among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Urine biosample					
Coleman et al., 2021, US (54)	See: Coleman et al., 2021	Non-pregnant: 0.15 (0.11-0.19) ng/mg creatinine Pregnant: 0.17 (0.04-0.67) ng/mg creatinine	0.17 (0.15-0.18) 0.882 0.15 (0.09-0.22) 1.133	0.17 (0.15-0.18) 0.882 0.22 (0.18-0.28) 0.773	NA
Dai et al., 2020, US (55)	See: Dai et al., 2020	Without symptoms: 0.2 (0.1-0.2) ng/mg creatinine With symptoms: 0.3 (0.2-0.4) ng/mg creatinine	0.2 (0.1-0.2) 1.0 0.2 (0.2-0.3) 1.5	NA	0.2 (0.1-0.2) 1.0 0.2 (0.2-0.2) 1.5

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**0.193 (0.165-0.225)**[c,d] **ng/mg creatinine**	**0.28 (0.256-0.305)** **0.689**	**0.277 (0.259-0.297)**[a] **0.697**	**0.149 (0.14-0.159)**[a] **1.295**
Perez et al., 2021, US (47)	See: Perez et al., 2021	**0.2 (0.1-0.2) ng/mg creatinine**	NA	**0.2 (0.2-0.2)**[d] **1.0**	**0.1 (0.1-0.1)**[c] **2.0**
Prokopowicz et al., 2020, Poland (121)	n = 88 Vapers' mean age: 28.8 (20-39), 48% females; dual users' mean (IQR) age: 26.2 (18-35), 69.2% females; smokers' mean (IQR) age: 28.1 (21-39), 44% females; non-users' mean (IQR) age: 28.9 (21-39), 37% females. Vapers (n=25): VP use for >6 months, smoked for >2 years and stopped smoking for >6 months, verified by CO. Dual users (n=13): TC smoking for >2 years and VP use for >6 months, verified by CO. Smokers (n=25): TC smoking for >2 years, verified by CO. Non-users (n=25): NR, verified by CO.	**Median (IQR): 0.29 (0.20-0.41) µg/g creatinine**	**0.26 (0.19-0.45)** **1.115**	**0.28 (0.20-0.51)** **1.036**	**0.35 (0.20-0.42)** **0.829**

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	**Dual users[b]**	**Smokers[c]**	**Non-users[d]**
Wiener et al., 2020, US (120)	n=1302 (metal analysis) 41% aged 26-44, 51.4% females, 61.5% non-Hispanic white. Vapers (n=NR): self-reported ever use of VP. Smokers (n=NR): self-reported current or former smoking with/without VP use. Non-users (n=680): self-reported never use of VP and smoking <100 TC in lifetime.	**0.11 (0.08-0.14) µg/L**	NA	**0.23 (0.20-0.27)[d]** **0.478**	**0.12 (0.11-0.14)[c]** **0.917**
Blood biosample					
Badea et al., 2018, Romania (119)	See: Badea et al., 2018	**Median (IQR): 0.03 (0; 0) ng/mL (BS)**	NA	**0.04 (0; 0.1)** **0.75**	**0.04 (0; 0)** **0.75**
Jain, 2019, US (67)	n = 1139 Sociodemographic characteristics NR. Vapers (n=52): self-reported VP use in past 5 days. Dual users (n=46): self-reported TC and VP use in past 5 days. Smokers (n=891): self-reported TC and/or cigars use in past 5 days. Non-users (n=176): no use of tobacco or nicotine products in past 5 days.	**0.30 (0.23-0.38)[b,c] µg/dL**	**0.81 (0.56-1.16)[a]** **0.370**	**0.80 (0.75-0.86)[a]** **0.375**	0.23 (0.22-0.24) 1.304

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Lee et al., 2020, South Korea (65)	n = 4744 Mean age among males: 45.7, mean age among females: 47.3, 54.4% females, mean (SD) BMI among males: 24.4 (0.1), mean (SD) BMI among females: 23.4 (0.1). Vapers (n=9): ex-smokers who used VP in past month. Dual users (n=57): smoking and VP use in past month. Smokers (n=926): smoking in past month. Non-users (n=2849): ex-smokers.	Geometric mean (SE): 0.8 (0.02) (B)	0.91 (0.08)	1.06 (0.02)	0.89 (0.01)
Prokopowicz et al., 2019, Poland (122)	n = 156 Vapers' mean age: 29.5, 50% females; dual users' mean age: 26.2, 41.4% females; smokers' mean age: 28.1, 53.6% females; non-users' mean age: 30.2, 54.9% females. Vapers (n=48): VP use for >6 months, smoked for >2 years and stopped smoking for >6 months. Dual users (n=29): TC smoking for >2 years and VP use for >6 months. Smokers (n=28): TC smoking for >2 years. Non-users (n=51): NR.	**0.44 (0.37-0.52)**[b,c,d] **µg/L**	**1.38 (1.11-1.72)**[a,d] **0.319**	**1.44 (1.16-1.78)**[a,d] **0.306**	**0.31 (0.26-0.36)**[a,b,c] **1.419**

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Lead

Lead is categorised as a possibly carcinogenic compound to humans according to the IARC (79) and as a carcinogen, respiratory toxicant, reproductive or developmental toxicant in humans by the FDA (80). It has a half-life of approximately 1-2 months in blood samples (methods: table 3) (36). Other than tobacco, there are many current (for example, industrial manufacturing) and historical sources (for example, leaded fuel and plumbing materials) of environmental exposures of lead that can contaminate soil, drinking water and air.

Four papers reported urinary levels of lead among vapers and smokers (36, 47, 54, 121) (table 40). Levels were reported to be between 10% (121) and 32% (36) lower among vapers compared to smokers, however these differences were not statistically significant. Perez and others found no difference in urinary lead levels between smokers and vapers (47). Colman and others reported urinary lead levels to be 30% lower among non-pregnant women who vape and 14% lower among pregnant women who vape when compared to smokers, however they did not test for statistical significance (54).

Four studies reported levels of lead in blood samples among vapers and smokers (65, 67, 119, 122). Levels were reported to be between 30% lower (120) and 4% higher (119) among vapers than smokers, however no study reported a statistically significant difference.

Four studies compared urinary levels of lead among vapers and non-users (36, 47, 55, 121). Two reported statistically significantly higher levels, between approximately 23% (36) and 33% (47), among vapers compared to non-users. Prokopowicz and others (121) reported urinary lead levels to be around 10% lower among vapers compared to non-users, however this was not statistically significant. Dai and others (55) found that vapers with self-reported respiratory symptoms had 25% higher levels of urinary lead than non-users with symptoms, but the study did not test for statistical significance. In comparison, vapers and non-users without self-reported respiratory symptoms, had similar urinary lead levels, but again the study did not test this comparison for statistical significance (55).

Four studies reported on blood levels of lead among vapers and non-users (65, 67, 119, 122). Among 3 studies, levels were reported to be between 11% (120) and 88% (119) higher among vapers than non-users, however differences were not statistically significant. Jain reported levels to be 2% higher among vapers but did not test for statistical significance (67).

Across cross-sectional studies that measured urinary lead, levels among vapers were approximately between 67% and 110% and levels among non-users were between approximately 69% and 113% of lead levels detected among smokers (figure 55).

Table 40. Cross-sectional studies reporting on levels of lead among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Urine biosample					
Coleman et al., 2021, US (54)	See: Coleman et al., 2021	Non-pregnant: 0.34 (0.29-0.39) ng/mg creatinine (U) Pregnant: 0.42 (0.17-1.0) ng/mg creatinine (U)	0.40 (0.35-0.45) 0.850 0.50 (0.35-0.71) 0.840	0.38 (0.35-0.41) 0.695 0.49 (0.42-0.57) 0.857	NA
Dai et al., 2020, US (55)	See: Dai et al., 2020	Without symptoms: 0.4 (0.3-0.4) ng/mg creatinine (U) With symptoms: 0.5 (0.4-0.7) ng/mg creatinine (U)	0.4 (0.4-0.4) 1.0 0.5 (0.4-0.5) 1.0	NA	0.4 (0.3-0.4) 1.0 0.4 (0.3-0.4) 1.25

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Goniewicz et al., 2018, US (36)	See: Goniewicz et al., 2018	**0.432 (0.382-0.488)[d] ng/mg creatinine (U)**	**0.5 (0.475-0.526)** **0.864**	**0.479 (0.462-0.496)** **0.902**	**0.351 (0.33-0.373)[a]** **1.231**
Perez et al., 2021, US (47)	See: Perez et al., 2021	**0.4 (0.3-0.4)[d] ng/mg creatinine (U)**	NA	**0.4 (0.4-0.4)[d]** **1.0**	**0.3 (0.3-0.3)[a,c]** **1.333**
Prokopowicz et al., 2020, Poland (121)	See: Prokopowicz et al., 2020	**Median (IQR): 0.66 (<LOD-1.14) µg/g creatinine (U)**	**0.3 (<LOD-0.91)** **2.2**	**0.98 (0.63-1.48)** **0.673**	**0.68 (<LOD-1.03)** **0.971**
Blood biosample					
Badea et al., 2018, Romania (119)	See: Badea et al., 2018	**Median (IQR): 2.24 (1; 3.5) ng/mL (BS)**	NA	**2.15 (1.1; 4.6)** **1.042**	**1.19 (0.9; 1.5)** **1.882**
Jain, 2019, US (67)	See: Jain, 2019	**0.90 (0.66-1.21) µg/dL (B)**	**1.18 (0.84-1.66)** **0.763**	**1.14 (1.07-1.21)** **0.789**	0.88 (0.84-0.92) 1.023

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	**Dual users[b]**	**Smokers[c]**	**Non-users[d]**
Prokopowicz et al., 2019, Poland (122)	See: Prokopowicz et al., 2019	**14.2 (12.5-16) µg/L (B)**	**13.9 (11.9-16.2)** **1.022**	**15.9 (13.9-18.6)[d]** **0.893**	**11.9 (10.6-13.3)[c]** **1.193**
Wiener et al., 2020, US (120)	n=1899 (lead analysis) 40.7% aged 26-44, 51% females, 61.7% non-Hispanic white. Vapers (n=NR): self-reported ever use of VP. Smokers (n=NR): self-reported current or former smoking with/without VP use. Non-users (n=1014): self-reported never use of VP and smoking <100 TC in lifetime.	**0.70 (0.64-0.77) µg/dL (B)**	NA	**1.0 (0.93-1.08)[d]** **0.700**	**0.63 (0.44-0.91)[c]** **1.111**

Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters ([a,b,c,d]) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Mercury

There is no evidence at present that exposure to mercury causes cancer in humans according to the IARC (79) however the FDA (80) classify it as a carcinogen, respiratory toxicant, reproductive or developmental toxicant in humans. It has a half-life of approximately 50 to 80 days (methods: table 3).

Two studies reported levels of mercury in blood samples (table 41). Badea and others reported inorganic mercury levels to be 10% lower among vapers compared to smokers (119). Jain reported total mercury levels to be 51% higher among vapers compared to smokers (67). Neither difference was reported to be statistically significant.

When vapers were compared to non-smokers, levels of mercury were reported to be between approximately 4% (119) and 17% (67) higher among vapers compared to non-users, however these were not statistically significant or were not tested for statistical significance.

Urinary levels of beryllium, chromium, cobalt, nickel, selenium, uranium and zinc were also reported in cross-sectional studies, (36, 55, 121, 123), however these metals are not on the WHO list of potential toxicants, therefore we did not report on them.

Table 41. Cross-sectional studies reporting on levels of mercury among vapers

Author, year, country	Participants' characteristics and grouping	Biomarker levels (biosample type[1]) and ratios between VP/DU and other conditions[2]			
		Vapers[a]	Dual users[b]	Smokers[c]	Non-users[d]
Blood biosample					
Badea et al., 2018, Romania (119)	See: Badea et al., 2018	Inorganic mercury **Median (IQR): 0.49 (0.5; 0.5) ng/mL (BS)**	NA	**0.55 (0.5; 0.6)** **0.891**	**0.47 (0.5; 0.5)** **1.043**
Jain, 2019, US (67)	See: Jain, 2019	Total mercury **0.98 (0.69-1.38) µg/dL (B)**	**0.65 (0.49-0.87)** **1.508**	**0.64 (0.58-0.71)** **1.531**	0.84 (0.78-0.90) 1.167

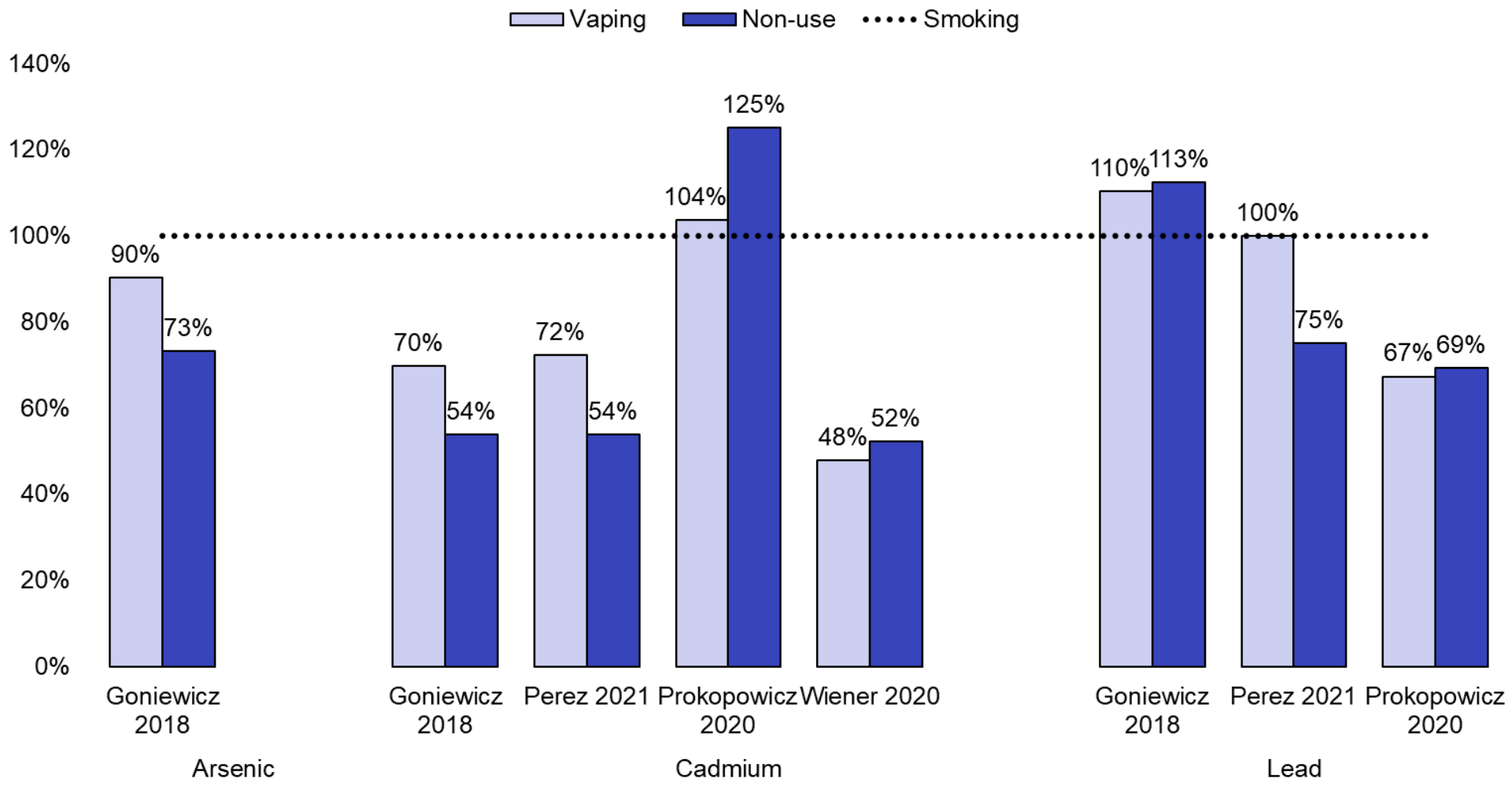
Notes: [1] Biosample types: B—blood, Br—breath, BP—blood plasma, BS—blood serum, H—hair, S—saliva, U—urine.

[2] Biomarker level ratios are calculated as (Levels in VP group)/(Levels in a comparison condition). A ratio below 1 indicates that a biomarker level in VP group was lower than in a comparison group while a ratio above 1 indicates how many times a biomarker level in VP group was higher than in a comparison group.

Bolded are participants' groups that were compared one to another in publications. Groups with different superscript letters (^{a,b,c,d}) were statistically significantly different from one another ($p<0.05$).

BMI—body mass index; IQR—inter-quartile range; LOD—level of detection; M—mean; NR—not reported; SD—standard deviation; TC—tobacco cigarette.

Figure 55. Levels of urinary metals in vapers and non-users relative to smokers



Summary of studies reporting on exposure to metals

Overall, there is inconsistency between studies assessing levels of metals among vapers in comparison to smokers and non-users, with some finding higher, similar or lower levels in vapers compared with smokers or non-users. Some metals, in the case of cadmium, have a very long half-life and can be influenced by many environmental exposures. Hence, a history of smoking will greatly affect the levels of metals among ex-smokers who vape. Cross-sectional research has limited control over extraneous variables and past use. This section focused on metals listed in the WHO list of priority toxicants (124), therefore has focused only on metals that are known to be potentially prevalent in tobacco.
Cross-sectional studies often did not collect information the type of device participants used; as the design features of vaping products have evolved over time, so have the metal components, which will likely also influence metal exposure. Conclusions cannot be confidently made on the level of exposure to metals from vaping products from this review.

7.9 Biomarkers of secondhand toxicant exposure

Study characteristics

Our literature search identified 6 studies (125-130) reporting on levels of potential toxicants in non-users exposed to secondhand vaping product aerosol (table 42). Two cross-over studies in total recruited 9 non-users who participated in 30 minute (125) and 2 hour (128) secondhand exposure sessions to vaping product aerosol in a room or a car. Two longitudinal studies (126, 127, 129) (Quintana and others reported findings from one study in 2 publications) explored effects of non-users' exposure to secondhand vaping product aerosol for up to 6 hours in 34 adults (129) and for a week in 52 children (126, 127). One cross-sectional study (130) measured urinary levels of NNAL, a metabolite of tobacco-specific nitrosamine NNK, in 55 non-users who reported living with vapers, smokers or other non-users of tobacco and nicotine products.

Risk of bias in included studies

Cross-over studies

Both cross-over studies that explored secondhand exposure to vaping product aerosol (125, 128) were assessed as having some concerns regarding overall risk of bias (appendices: table 2). Key limitations of these studies were associated with concerns regarding randomisation process and a lack of pre-registered analysis plan.

Longitudinal studies

Three publications reporting on 2 non-randomised longitudinal studies (126, 127, 129) were assessed at having serious risk of bias (appendices: table 3). Johnson and others (129) was assessed at serious risk of bias due to the selection of participants—participants non randomly attended 4 public vaping convention events with some of them attending 2 or more events. Quintana and others (126, 127) was assessed at serious risk of bias due to classification of exposure—the study explored at-home exposure to tobacco smoke or vaping product aerosol that is difficult to control and compare between different households.

Cross-sectional study

Risk of bias of one cross-sectional study (130) was assessed using Biocross quality appraisal tool (appendices: table 4). Key limitations of this study were associated with lack of information about representativeness of the study population and lack of discussion about the study limitations due to its cross-sectional nature.

Study findings

Nicotine

A cross-over study by Amalia and others reported on salivary nicotine, cotinine and 3-hydroxycotinine levels in non-users after 30 minutes exposure to secondhand vaping product aerosol in 2 settings, an office room and a car (125) (table 42). Only salivary cotinine levels were above limit of detection (0.05 ng/mL) immediately after exposure to secondhand vaping product aerosol in the office room condition, while measures of salivary nicotine and salivary 3-hydroxycotinine were below the limit of detection in both conditions. Another cross-over study by Melstrom and others (128) assessed changes in blood, salivary and urinary levels of cotinine in non-users who were exposed to 2-hour vaping sessions by 3 vaping product users. Authors reported median increase in cotinine levels across all biosamples compared with baseline, and the increase was larger when vaping product users used tank rather than disposable type vaping products (128).

A longitudinal study by Johnson and others (129) assessed changes in urinary and salivary cotinine and in urinary 3-hydroxycotinine among non-users who attended 4 vaping convention events, where they were exposed to secondhand vaping product aerosol for up to 6 hours (table 42). Authors concluded that levels of nicotine metabolites among non-users increased statistically significantly from 2- to 13-fold dependent on the event and peaked around 4 hours after 6-hour exposure sessions (129). A longitudinal study by Quintana and others (126, 127) measured urinary cotinine levels in children exposed to smoking and vaping in their home environment. Urinary cotinine levels were statistically significantly higher in children who lived with at least one smoking adult compared with children who lived with vaping product users or non-users; cotinine levels were also

statistically significantly higher in wristbands of those children who lived at home with vaping product using adults compared with children who lived with non-smoking and non-vaping adults (126, 127).

Table 42. Studies reporting on secondhand exposure to nicotine and its metabolites due to vaping

Author, year, country	Study design, exposure length	Baseline participants' characteristics and grouping/assignment	Results
Nicotine			
Amalia et al., 2021, Spain (125)	Cross-over, Single 30 minutes exposure (A)	n = 3 One vaper and 2 non-users—adult never users of TC or VP or had stopped using >6 months ago. Non-users: male (aged 49) and female (aged 40), both Caucasians. Vaper: 59 year old Caucasian female had used a VP daily for 3.5 years. Non-users were exposed to two secondhand exposure sessions (the office room of 35.2 m3 and the car of 10 m3) separated by 10-day washout period. Exposure was to secondhand vapour from ad lib vaping of a modular VP (Eleaf iStick, 40W, 2600 mAh battery, 220°C, 1 Ω coil) with 50%/50% PG/VG, cinnamon cookie flavoured and 3 mg/mL nicotine strength e-liquid. Baseline sample taken 5 mins before exposure FUs: 5 minutes after exposure, 30 min and 180 min post-exposure.	Saliva < limit of detection (0.50 ng/mL) during all conditions and at all FUs.

Author, year, country	Study design, exposure length	Baseline participants' characteristics and grouping/assignment	Results
Cotinine			
Amalia et al., 2021, Spain (125)	Cross-over, Single 30 minutes exposure (A)	See: Amalia et al., 2021	Saliva After exposure: 8 out of 10 measurements of cotinine after exposure in the room were higher than limit of detection (0.05 ng/mL). < limit of detection during all other FUs at both conditions.
Melstrom et al., 2018, US (128)	Cross-over, 2 hours (A)	n = 9 (6 non-users and 3 vapers) Non-users (n=6): self-reported never use of TC (<100 TC in their lifetime) and no use of other products (incl. NRT) in the past year. Aged 28-54, 2 females, 4 white and 2 African American. Two 2-hour sessions included all participants in closed 52.6 m^3 room with furniture. Vapers were ad lib using their own-brand disposable VP (first session, mean nicotine strength 16.4 mg/mL) and tank VP (second session, mean nicotine strength 15.1 mg/mL). FUs: during exposure and during the 6-hour FU period.	Median increase in cotinine levels within non-users' group compared with baseline: Blood serum Disposable VP: 0.007 ng/mL Tank VP: 0.041 ng/mL Saliva Disposable VP: 0.033 ng/mL Tank VP: 0.060 ng/mL Urine Disposable VP: 0.316 ng/mg creatinine Tank VP: 0.948 ng/mg creatinine
Quintana et al., 2019 & 2021, US (126, 127)	Longitudinal, 7 days (A)	n = 53 Children who did not smoke or use VPs. Children's age groups: 3 to <6 years: n=12 (23%) 6 to <11 years: n=28 (53%) 11 to 14 years: n=13 (25%) 60.4% females, 40% multi-racial, 23% Latinx, 21% white, 13% black, 4%	Urine (7 days) Stat. sig. higher in smokers' environment compared with vapers and non-users' environment (p<0.01). Stat. sig. higher in vapers' environment compared with non-users' environment (p<0.01).

Author, year, country	Study design, exposure length	Baseline participants' characteristics and grouping/assignment	Results
		Asian/Pacific islander. Children were grouped to: 1) Vaping environment (n=19): children who lived with at least one adult who used a VP with nicotine e-liquids for >3 days a week. 2) Smoking environment (n=19): children who lived with at least one adult who smoked >6 TC per week at home. 3) Non-use environment (n=15): children who lived with adults who did not smoke or use VP and had a ban on inside smoking/vaping.	
Johnson et al., 2019, US (129)	Longitudinal, Single exposure (A)	n = 34 Non-users: not a current tobacco or nicotine user. Age range: 19-30, 68% females. Participants attended four vaping convention events where they were exposed to secondhand VP vapour: Event 1: n=10, ~1000 attendees, 341-351 exposure minutes. Event 2: n=9, ~300 attendees, 350 exposure minutes. Event 3: n=11, ~150 attendees, 340 exposure minutes. Event 4: n=4, ~1500 attendees, 360-363 exposure minutes.	Urine Stat. sig. diff. across sampling times ($p<0.0001$), events ($p<0.0001$) and interaction between events and sampling times ($p<0.05$). Adjusted mean ratio (max/baseline) by events: Event 1: 8.14 Event 2: 6.77 Event 3: 2.67 Event 4: 13.16 Saliva Adjusted mean ratio (max/baseline) by events: Event 1: 4.58 Event 2: 7.07 Event 3: 2.02 Event 4: 12.68

Author, year, country	Study design, exposure length	Baseline participants' characteristics and grouping/assignment	Results
		FUs: post-exposure, 4 hours post-exposure, next morning after exposure.	
3-hydroxycotinine (3-HC)			
Amalia et al., 2021, Spain (125)	Cross-over, Single 30 minutes exposure (A)	See: Amalia et al., 2021	Saliva < limit of detection (0.04 ng/mL) during all conditions and at all FUs.
Johnson et al., 2019, US (129)	Longitudinal, Single exposure (A)	See: Johnson et al., 2019	Urine Stat. sig. diff. across sampling times ($p<0.0001$), events ($p<0.0001$) and interaction between events and sampling times ($p<0.05$). Adjusted mean ratio (max/baseline) by events: Event 1: 6.84 Event 2: 5.68 Event 3: 2.24 Event 4: 8.79

Notes: A—acute exposure; FU—follow-up; stat. sig. diff.—statistically significant difference.

Acrolein

A longitudinal study by Johnson and others (129) assessed changes in urinary levels of acrolein metabolites (3-HPMA and CEMA) (table 43). Authors concluded that 6-hour exposure to secondhand vaping product aerosol statistically significantly increased non-users' exposure to acrolein from 16% to 282% dependent on the vaping convention event they attended (129).

Table 43. Studies reporting on secondhand exposure to biomarkers of acrolein due to vaping

Author, year, country	Study design, exposure length	Baseline participants' characteristics and grouping/assignment	Results
3-HPMA			
Johnson et al., 2019, US (129)	Longitudinal, Single exposure (A)	See: Johnson et al., 2019	Urine Stat. sig. diff. across sampling times ($p<0.0001$), events ($p<0.0001$) and interaction between events and sampling times ($p<0.05$). Adjusted mean ratio (max/baseline) by events: Event 1: 3.82 Event 2: 1.28 Event 3: 2.18 Event 4: 1.83
CEMA			
Johnson et al., 2019, US (129)	Longitudinal, Single exposure (A)	See: Johnson et al., 2019	Urine Stat. sig. diff. across sampling times ($p<0.01$) but not across sampling events. Adjusted mean ratio (max/baseline) by events: Event 1: 2.40 Event 2: 1.82 Event 3: 1.92 Event 4: 1.16

Notes: A—acute exposure; FU—follow-up; stat. sig. diff.—statistically significant difference.

Tobacco-specific nitrosamines (NNAL, NNK, NAB, NAT, NNN)

A cross-over study by Amalia and others (125) did not detect salivary NNK, NNAL and NNN after 30-minute secondhand exposures to vaping product aerosol (table 44).

Johnson and others (129) reported that nearly all urinary samples of their participants were below the limit of detection for NNAL, NAB, NAT and NNN concentrations (table 44).

A cross-sectional study by Martínez-Sánchez and others (130) reported on urinary NNAL levels in non-users who lived with smokers, vapers or other non-users (table 44). More than a half of participants' NNAL levels were below the limit of detection. Among participants whose NNAL levels were measured, authors found statistically significantly higher median urinary NNAL levels in those who lived with smokers compared with those who lived with non-smokers.

Table 44. Studies reporting on secondhand exposure to biomarkers of tobacco-specific nitrosamines due to vaping

Author, year, country	Study design, exposure length	Baseline participants' characteristics and grouping/assignment	Results
NNAL (NNK)			
Amalia et al., 2021, Spain (125)	Cross-over, Single 30 minutes exposure (A)	See: Amalia et al., 2021	Saliva NNK < limit of detection (2.0 pg/mL) during all conditions and at all FUs. Saliva NNAL < limit of detection (0.50 pg/mL) during all conditions and at all FUs.
Johnson et al., 2019, US (129)	Longitudinal, Single exposure (A)	See: Johnson et al., 2019	Urine 84% of samples were below the limit of detection. 38% of detected NNAL concentrations were in pre-exposure samples collected from participants before event 1 (n=1), event 2 (n=3) and event 3 (n=2).
Martínez-Sánchez et al., 2019, Spain (130)	Cross-sectional	n = 55 Non-users: self-reported no use tobacco or NRT and are not exposed to smoke at work, transport or during leisure. Sociodemographics NR. Participants live with: Vapers (n=6) Smokers (n=25) Non-users (n=24)	Urine % (n) of detectable NNAL and median NNAL in groups: Living with vapers: 66.7% (n=4), 0.55 pg/mL Living with smokers: 76% (n=19), 0.46 pg/mL Living with non-users: 29.2% (n=7), 0.33 pg/mL Stat. sig. higher in those living with smokers compared with those living with non-users (p=0.017). NS diff. between other groups.

Author, year, country	Study design, exposure length	Baseline participants' characteristics and grouping/assignment	Results
NNN			
Amalia et al., 2021, Spain (125)	Cross-over, Single 30 minutes exposure (A)	See: Amalia et al., 2021	Saliva < limit of detection (1.0 pg/mL) during all conditions and at all FUs.
Johnson et al., 2019, US (129)	Longitudinal, Single exposure (A)	See: Johnson et al., 2019	Urine Below the limit of detection in all samples for all sampling times and sampling events.
NAB			
Johnson et al., 2019, US (129)	Longitudinal, Single exposure (A)	See: Johnson et al., 2019	Urine Below the limit of detection in all samples for all sampling times and sampling events.
NAT			
Johnson et al., 2019, US (129)	Longitudinal, Single exposure (A)	See: Johnson et al., 2019	Urine Below the limit of detection in all samples for all sampling times and sampling events.

Notes: A—acute exposure; FU—follow-up; stat. sig. diff.—statistically significant difference.

Summary of studies reporting on secondhand exposure to vaping product aerosol

Based on the evidence from limited number of studies, secondhand exposure to vaping product aerosol might increase non-users' exposure to potential toxicants. However, studies that reported statistically significant increase in nicotine or biomarkers of volatile organic compounds in non-users usually overexposed them to secondhand vaping product aerosol—for example, non-users stayed for 2 hours in a room with 3 vaping product users (128) or for 6 hours in an indoor vaping convention event with 150 or 1500 vapers (129). Shorter exposures to secondhand vaping product vapour in confined spaces did not result in detectable levels of nicotine, volatile organic compounds, or tobacco-specific nitrosamine metabolites.

7.10 Conclusions

This chapter examined findings from our systematic review on biomarkers of nicotine and potential toxicants relevant to our review protocol questions—first, the effect of vaping and secondhand exposure to vaping products that are associated with the risk of health conditions, and secondly the effects of vaping among people with existing health conditions on disease outcomes. However, we identified no study addressing the second review question—only one study assessed participants with self-reported respiratory symptoms and did not test for statistical differences across relevant groups. Hence our review for this chapter is confined to our first review question. We assessed both relative and absolute vaping risks associated with exposure to nicotine and potential toxicants where the data were available (that is, between vapers and smokers, and between vapers and non-users), and where feasible included comparisons across different population groups.

The included studies used a range of different designs and had varying quality or risk of bias.

The studies we have included used a range of different definitions of vaping and smoking. For example, findings of some studies were confounded by treating vapers who smoke, occasional vapers and/or exclusive daily vapers as a uniform group or comparing occasional vapers with daily smokers. Hence findings need to be cautiously interpreted.

Studies looking at participants at more than one time point mostly explored acute exposure to vaping or followed-up participants for short- to medium-term. So, we were unable to summarise findings on longer term vaping exposure, with some studies not allowing adequate wash-out periods for biomarkers with longer half-lives.

In line with our algorithm, we carried out meta-analyses wherever possible, but a lack of consistency in study designs, biomarker reporting, group definitions and exposure periods resulted in few studies being included. Studies funded by the tobacco industry, which were included in the meta-analyses, used a consistent methodology although their follow ups are of limited duration and their findings pertain to switching from smoking to vaping in confinement.

Here we summarise our findings for each biomarker for relative and absolute differences in various populations of interest, starting with first-hand vaping exposure.

Nicotine

There was substantial variation across the 60 studies included in this section, with only 5 (4 longitudinal and one cross-sectional) were from the UK. Levels of nicotine and nicotine metabolites in participants using vaping products differed according to study design, definitions of vaping and smoking, biomarker and biosample used, and exposure duration.

To assess relative exposures between vaping and smoking, we were able to carry out 5 meta-analyses of nicotine and nicotine metabolites (one longitudinal, 4 cross-sectional) among people who vaped and smoked at least weekly. All found no significant differences across the groups. From the narrative summaries, evidence suggests that over time and with increased experience of vaping, users can derive similar levels of nicotine as they can from smoking cigarettes. Levels of nicotine metabolites varied with vaping device characteristics (for example, vaping device types, e-liquid nicotine concentrations).

To assess absolute exposures between vapers and non-users, we were able to carry out 4 meta-analyses of nicotine biomarkers which, as expected, showed significantly higher levels among vapers than non-users. In general findings from the narrative summaries were similar for absolute nicotine exposures.

There were no discernible differences between adults and adolescent exposures to nicotine and its metabolites.

Volatile organic compounds

Twenty-four studies assessed VOCs, with only 5 from the UK. Again, there was considerable variation across the studies in terms of design, definitions of vaping and smoking, biomarker measurements and exposure duration.

To assess relative exposures between vaping and smoking, we were able to carry out 15 meta-analyses of VOCs (4 longitudinal, 11 cross-sectional). Findings varied by biomarker. In general, most showed statistically significantly lower levels of VOCs among vapers than smokers, with substantive reductions in some biomarkers, such as the acrolein metabolite

3-HPMA, the acrylonitrile metabolite CYMA and 1,3-Butadiene metabolite MHBMA. For a few VOCs, such as formaldehyde and toluene, available evidence was inconclusive regarding significant differences between vaper and smokers.

To assess absolute exposures between vapers and non-users, we were able to carry out 10 meta-analyses (all cross-sectional). All showed no significant differences between vapers and non-users, except for the acrylonitrile metabolite CNEMA. One study showed that average levels for vapers were over 3 times higher than those among non-users.

In general, findings from the narrative summaries were similar for absolute and relative VOC exposures.

Levels among young people were broadly in the same direction to levels reported among adults, with some differences for individual biomarkers, which may be due to different smoking and vaping patterns.

Tobacco specific nitrosamines

Twenty-eight studies assessed TSNAs, with only 3 from the UK. As for other biomarkers, there was considerable variation across the studies in terms of design, definitions of vaping and smoking, biomarker measurements and exposure duration.

To assess relative exposures between vaping and smoking, we were able to carry out 5 meta-analyses of TSNAs (2 longitudinal, 3 cross-sectional). These all showed significantly lower levels of TSNAs among vapers than smokers, with substantially lower levels for NNAL, NAB, NAT and NNN. Findings were generally consistent with those reported in the narrative summaries.

To assess absolute exposures between vapers and non-users, we were able to carry out 3 meta-analyses using cross-sectional data, which all showed significantly higher levels of TSNAs among vapers than non-users. However, the cross-sectional data make it difficult to distinguish exposure from vaping products from previous tobacco use. Furthermore, evidence from an RCT and a cross-over study indicates that TSNA metabolite levels among vapers might decrease to a similar level as among non-users.

Levels among young people were in the same direction as among adults, although the magnitude of difference between vapers and smokers was substantially less for young people compared with adults. Again, this may be due to different smoking and vaping patterns among adults and young people.

Other potential toxicants

Nine studies assessed a range of other potential toxicants, such as polyaromatic hydrocarbons, with only one from the UK. We were unable to carry out any meta-analyses. Generally, the very limited findings suggested the levels of these other potential toxicants were lower among vapers than smokers, and higher among vapers than non-users.

Carbon monoxide

Thirty-three studies assessed carbon monoxide exposure, with 3 studies from the UK. As for other biomarkers, there was considerable differences in methods across the studies and user definitions.

To assess relative exposures between vaping and smoking, we carried out 2 meta-analyses. Both showed significantly lower blood carboxyhaemoglobin levels among vapers than smokers.

We were unable to carry out any meta-analyses of exposures between vapers and non-users. But some interventional studies suggested that exposure to CO in smokers who completely switch to vaping product use might be reduced to levels similar to non-users.

Metals

Ten cross-sectional studies examined a range of metals (arsenic, cadmium, lead, mercury), with none from the UK. No meta-analyses could be carried out.

In general, the studies had mixed findings about relative exposure.

Absolute exposure assessments were also mixed although most studies showed higher levels of exposure among vapers than non-users.

Secondhand exposure

Six studies assessed secondhand exposure to vaping product aerosol, using a variety of biomarkers, none from the UK. The level of exposure varied greatly from people at home to people attending an indoor vaping convention.

Short exposures to secondhand vaping did not result in detectable changes in levels of nicotine, VOCs or TSNAs. However, longer exposures during heavy sustained vaping were associated with significant increases in nicotine or potential toxicants' metabolites.

7.11 Implications

Our systematic review covered a wide range of biomarkers and studies. Findings are broadly consistent with the few previous reviews in this area, but because of the greater volume of research that has been conducted in recent years, the implications are much clearer. The reviewed studies show that compared to smoking, use of vaping products leads to a substantial reduction in biomarkers of toxicant exposure associated with cigarette smoking. However, the degree of any residual risk remains unclear, mainly because of the lack of comparisons of long-term former smokers who do and do not vape or comparisons with those who have never smoked or vaped.

Our quality assessments revealed most studies had some methodological concerns, and these should be addressed in future research as they limit interpretations of our findings. For example, a lack of significant differences between levels of exposure between people who vape and non-users may be due to background environmental exposures, a lack of sensitivity in biomarker measurement methods or because exposure to potential toxicants between people who vape and non-users is relatively similar.

Historical tobacco use can greatly affect many of the biomarkers used to determine exposure to potentially harmful constituents from vaping. So, as most vapers are previous long-term smokers (see chapter 4 on vaping among adults), strict definitions for duration of exclusive vaping should be used consistently in future studies. Similarly, definitions should preclude concurrent smoking, and only include people who exclusively vape. This is particularly important for cross-sectional studies, but longitudinal studies should also use objective measurements to assess concurrent cigarette smoking. Future studies should always verify biologically participants' smoking, vaping or non-use status, rather than rely on self-reports. Based on our review findings, measurements of carbon monoxide or NNAL could be used to improve over-reliance on self-reported vaping and smoking.

More research is needed on biomarkers of exposure among vapers, particularly in the UK, where we identified a lack of studies. We would encourage research with longitudinal and cross-sectional designs. While longitudinal research is more robust, particularly in relation to changes over time, cross-sectional research also offers insight into exposure from realistic and naturalistic use patterns. Longitudinal research would benefit from including longer follow-up periods to be able to assess long-term changes in biomarker exposure among vapers who sustain use over long periods of time (see chapter 4 on vaping among adults). This is also important for biomarkers with longer half-lives. In our meta-analyses, many findings were from tobacco industry funded RCTs conducted in confinement for periods of up to 7 days. So, future research needs to include more independent research of biomarkers of exposure in people who use vaping products, smoke and do not use tobacco or nicotine outside of confinement, and with longer follow-ups.

Several biomarkers of exposure are not specific to tobacco, and almost all biomarkers are susceptible to the effects of confounders. For example, VOCs are prevalent in many household products such as paints and cosmetics and can also be influenced by diet. The local environment can also uniquely influence exposure, with higher levels of PAHs and other toxicants found in urban environments due to motor vehicle exhaust fumes and other sources of pollution. Different toxicant exposures are also present in rural environments, due to pesticide exposure and other agricultural pollutants. So, strict control for confounders and large sample sizes are needed to reduce the influences of other environmental exposure on findings in cross-sectional research.

Our systematic review used the WHO priority toxic contents and emissions list for tobacco products. Biomarkers of exposure should instead be tailored for vaping products, and there are already suggestions to include vaping specific biomarkers in the WHO list which will help guide future research. Due to the variety of different metal elements used for vaping product components, there may be exposure to certain metals from vaping that are not present in exposure from tobacco. Future research is needed to identify types of metal exposure which are exclusively from vaping products and how these can be mitigated.

There is a lack of comparable research on biomarkers of exposure to nicotine and potential toxicants across different population groups, such as young people and adults, participants from different gender, ethnicity or socioeconomic status groups.

Given we identified no studies assessing the biomarkers of exposure to vaping among people with existing health conditions on disease outcomes, this is an important gap that should be addressed by funding bodies.

Overall, despite the methodological limitations identified in our systematic review, evidence suggests significantly lower relative exposure from vaping compared to smoking in biomarkers that are associated with the risk of cancer, respiratory, cardiovascular or other health conditions. This is consistent with encouraging people who smoke to use vaping products to stop smoking or as alternative nicotine delivery devices. Also, our findings of higher absolute exposure from vaping compared with not using any nicotine products reinforce the need to discourage never smokers from taking up vaping (or smoking).

7.12 References

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8 Biomarkers of potential harm to health cutting across several diseases

8.1 Introduction

Biomarkers of potential harm have been defined as ‘the measurement of an effect due to exposure, these include early biological effects, alterations in morphology, structure or function, and clinical symptoms consistent with harm; these include preclinical changes’ (1). In subsequent chapters on cancers, cardiovascular, respiratory and other diseases we discuss biomarkers of potential harm that have been shown to increase the risk of those diseases. The objective of this chapter is to summarise the evidence from the included studies that report on associations of exposure to vaping with biomarkers of potential harm that cut across several diseases (table 1). Information about these biomarkers is summarised in the methods chapter (table 4) under oxidative stress, inflammation, endothelial function and other marker categories. We will refer to these specific biomarkers of potential harm to health when discussing the other disease-specific outcomes in subsequent chapters.

Summary of previous reports about the effect of vaping on biomarkers of oxidative stress, inflammation, endothelial and platelet function

In line with other chapters, first we summarise the evidence from our previous evidence reviews of vaping products (2-6), the Committee on Toxicity (COT) report on toxicological risks from vaping products (7) and the National Academies of Science, Engineering and Medicine (NASEM) review of vaping products (8).

Our previous Vaping in England evidence review reports commissioned by Public Health England did not review evidence on biomarkers of oxidative stress, inflammation, endothelial and platelet function (methods: table 4). The COT report (7) considered evidence from several clinical studies that reported on vaping effects on oxidative stress and inflammation but did not exclusively summarise findings regarding oxidative stress or inflammation biomarkers. Nevertheless, most of the publications that were considered by the COT, were also included in our systematic literature review.

The NASEM report (8) emphasised that existing evidence on smoking-induced effects on endothelial cell dysfunction and oxidative stress damage warrants investigation into how vaping products compare with smoking and non-use of tobacco and nicotine products regarding these health risks. Therefore, the NASEM review presented possible ways that vaping might affect endothelial function and induce oxidative stress, and reviewed available evidence published up to the end of August 2017 (8).

Most reviewed evidence was from cell and animal studies, and only 2 studies reported on the effects of vaping on biomarkers of potential harm in human participants. A study by Antoniewicz and others (9) reported that acute exposure to vaping product use resulted in increased levels of circulating endothelial progenitor cells but did not change the levels of microvesicles. The authors concluded that the measured effect of vaping on endothelial progenitor cells might be indicative of vascular injury similar to the effect produced by smoking. Another cross-sectional study by Carnevale and others (10) compared changes in oxidative stress markers after ad libitum vaping or smoking for a week. The study found increased levels of soluble Nox2-derived peptide and 8-iso-prostaglandin F2α and significantly decreased nitric oxide and vitamin E levels. Furthermore, there was a statistically significant reduction in flow-mediated dilation function after participants vaped or smoked for a week. The authors concluded that both smoking and vaping induced the oxidative stress, but the use of vaping products appeared to produce a less pronounced effect on levels of soluble Nox2-derived peptide, 8-iso-prostaglandin F2α and nitric oxide than cigarette smoking. Both human studies explored a relatively short exposure to vaping effects, and Carnevale and others (10) noted that future research should clarify the chronic vascular effects of vaping product use.

The NASEM report also reviewed multiple cell and animal studies on vaping-induced endothelial dysfunction and oxidative stress and noted significant methodological heterogeneity between the studies. For instance, comparisons between cell studies were difficult due to different cell cultures used, varying exposure methods (for example, cells were exposed to vaping e-liquids, aerosol extract or aerosol generated directly by vaping products), and different lengths of exposure. Based mainly on findings from cell and animal studies, NASEM concluded that:

> "There is substantial evidence that e-cigarette aerosols can induce acute endothelial cell dysfunction, although the long-term consequences and outcomes on these parameters with long-term exposure to e-cigarette aerosol are uncertain" (8).

> "There is substantial evidence that components of e-cigarette aerosols can promote formation of reactive oxygen species/oxidative stress. Although this supports the biological plausibility of tissue injury and disease from long-term exposure to e-cigarette aerosols, generation of reactive oxygen species and oxidative stress induction are generally lower from e-cigarettes than from combustible tobacco cigarette smoke" (8).

Next, we present findings from our systematic literature review on biomarkers of potential harm to health that cut across multiple health systems.

8.2 Study characteristics

Our literature search identified 41 unique studies (reported in 43 publications) which assessed biomarkers of potential harm associated with oxidative stress, inflammation, endothelial function and platelet activation biomarkers. Two of these studies were randomised controlled trials (RCTs) (11, 12), 11 were cross-over trials (13-23), 6 were non-randomised longitudinal studies (16, 24-29) and 24 were cross-sectional studies (30-53). One publication (16) reported findings from cross-over and longitudinal study phases and 4 publications reported findings from 2 studies—2 from the same longitudinal study (26, 27), and 2 from the same cross-sectional study (47, 48).

Of the 43 publications, 3 were conducted in the UK (11, 17, 24), 22 in the US (12-15, 20, 23, 26, 27, 29, 31, 33-35, 38-41, 43-49), 3 in Greece (16, 25, 51), Italy (18, 19, 21) and Saudi Arabia (36, 52, 53), 2 in Turkey (37, 42) and South Korea (30, 32) and one in Egypt (50), Germany (28) and Sweden (22). Two studies reported funding from the tobacco industry (24, 35) and authors of one study reported receiving funding from vaping product companies (37) (appendices: table 5).

Sample sizes of the included studies ranged from 10 in an acute exposure study (29) to 7,505 in a cross-sectional study which used the Korean National Health and Nutrition Examination Survey (KNHANES) data (30). Participants' mean age ranged from 24 in a cross-over study (23) to 51.7 in a cross-sectional study (39) and between 22% (14) and 80% (25) of participants were females. Three studies included all male participants (17, 30, 32) and one included all female participants (31). Also, 2 studies recruited participants with a diagnosis of asthma (50, 51) and 3 included participants with dental implants or a diagnosis of periodontal disease (42, 52, 53).

RCTs

A total of 148 participants were recruited across the 2 RCTs (11, 12) that assessed oxidative stress, inflammation, endothelial function and other biomarkers of potential harm. George and others recruited smokers of at least 15 cigarettes per day and randomised them to vaping with nicotine, vaping without nicotine or continued smoking groups. Song and others recruited healthy, young non-users (age range 21 to 30) who were randomised to ad libitum vaping e-liquid with propylene glycol and vegetable glycerine but without nicotine and flavourings or no use of vaping products. Both RCTs followed up their participants for 4 weeks.

Cross-over studies

Across the 11 cross-over studies (13-23), 450 participants were recruited. Four studies recruited only smokers—those attending a smoking cessation unit (16), smoking on average for 15 years (21), smoking at least one cigarette per day (17) and smoking less

than 11 cigarettes per month (22). Two studies recruited ‘dual users’ smoking more than 5 (14) and more than 10 cigarettes per day (13), and 2 studies recruited non-users of tobacco and nicotine products (20, 23). The remaining studies included participants with different smoking or vaping statuses—Haptonstall and others recruited vapers, smokers and non-users (15), Mastrangeli and others recruited vapers and smokers (18) and Nocella and others recruited smokers and non-users (19). Three studies recruited non-users who were exposed to using a vaping product with and without nicotine (20, 23) or using a vaping product and smoking a tobacco cigarette (19). Most cross-over studies included acute exposure conditions of using a vaping product with or without nicotine, smoking, using heated tobacco products (HTP), using nicotine inhalers or not using tobacco and nicotine products. The 2 studies that recruited ‘dual users’ had 48 hours (14) and 5 days (13) cross-over conditions.

Longitudinal studies

Six non-randomised longitudinal studies reported in 7 publications (16, 24-29) in total recruited 380 participants. Four studies recruited smokers only (16, 24, 25, 28), one study recruited never smokers (29), and a study, which was reported in 2 publications (26, 27), recruited non-users who were exposed to a single session of vaping product use without nicotine. Other studies compared outcomes between participants who switched from smoking to vaping product use, dual use or continued smoking. Three studies tested changes after acute exposure to vaping product use (26-29), one study, which also included a cross-over study phase, followed-up participants for a month (16), one for 4 months (25) and a study funded by the tobacco industry reported findings 24 months after participants switched from smoking to vaping product use (24).

Cross-sectional studies

A total of 13,007 participants were recruited across the 24 cross-sectional studies that assessed oxidative stress, inflammation, endothelial function and other biomarkers of potential harm to health (30-53). Across the cross-sectional studies, participants were grouped as vaping product users, ‘dual users’, smokers and non-users of nicotine or tobacco products based on self-report with varied definitions for inclusion.

Two cross-sectional studies used the same data from the 7th KNHANES conducted in 2016 (30, 32); Kim and others also included data from the 6th KNHANES. Perez and others used data from wave 1 of the Population Assessment of Tobacco and Health (PATH) survey (31).

8.3 Risk of bias in included studies

RCTs

Both RCTs were assessed to have some concerns in relation to overall risk of bias according to the RoB2 risk of bias tool (appendices: table 1). Key concerns regarding risk of bias of these RCTs were related with a lack of information on the randomisation process, deviations from intended interventions and lack of pre-specified data analysis plans.

Cross-over studies

Of the 11 cross-over studies that reported on biomarkers of potential harm to health, 10 were rated at some concerns of risk of bias (13-15, 17-23) and one at high risk of bias (16) according to the RoB2 risk of bias tool for cross-over studies (appendices: table 2). The high risk of bias assessment for Ikonomidis and others study (16) was due to short 60-minute washout period between cross-over conditions.

Longitudinal studies

Of the 6 longitudinal studies, one was rated at low risk of bias (29), 4 at moderate risk of bias (24-28) and one was rated at serious risk of bias (16) due to deviations from intended interventions according to the ROBINS-I risk of bias tool for non-randomised longitudinal studies (appendices: table 3).

Cross-sectional studies

The quality of all cross-sectional studies was assessed using Biocross quality appraisal tool and is reported in appendices (appendices: table 4). The included studies were rated from 5 to 16 out of 20 in terms of their risk of bias. The main limitations were associated with study population representativeness (for example, lack of sampling frame definition, sample size justification or information about response rate) and lack of discussion on limitations arising from the cross-sectional study design.

8.4 Study findings

Next, we report findings from the included studies on 4 groups of biomarkers of potential harm to health—oxidative stress, inflammation, endothelial function and platelet activation. We followed the algorithm for selecting studies for meta-analysis (methods: table 6) and, where study characteristics and reported findings allowed, pooled data across studies using meta-analysis.

Oxidative stress

Tobacco smoke is one of the many known factors influencing the oxidative stress on human body. The oxidative stress is known to increase with age (54), men are more susceptible to oxidative stress than women (55) and other factors, like genetics, diet, air pollution and physical activity, are known to affect the oxidative stress and its biomarkers.

The included studies reported on vaping association with a range of oxidative stress biomarkers that we had identified based on the US FDA sponsored workshop on biomarkers of potential harm (56) except for triglycerides or blood serum levels of vitamin C. Next, we discuss findings on the oxidative stress biomarkers.

Oxidative stress biomarkers

Oxidised low-density lipoprotein (LDL)

Levels of oxidised low-density lipoprotein (LDL) is one of the blood lipid profile indicators that can contribute to the development of cardiovascular diseases and atherosclerosis (56). Tobacco smoking (as well as diet, physical activity and genetics) is associated with increased LDL levels.

High-density lipoprotein (HDL)

Tobacco smoking is related with decreased high-density lipoprotein levels (HDL) in blood. HDL levels are also inversely related to cardiovascular diseases and are known to be associated with diet, physical activity and genetics.

8-isoprostane (8-iso-prostaglandin F2α)

8-isoprostane is a marker of antioxidant deficiency and its blood levels tend to increase after smoking. The elevated 8-isoprostane levels are associated with multiple diseases (57), including cancers, cardiovascular and lung diseases (58).

Soluble Nox2-derived peptide (sNOX2-dp)

A marker of Nox2 activation detected in blood, which also produces reactive oxygen species (see below).

Malondialdehyde (MDA)

Malondialdehyde is a biomarker of oxidative damage to lipids detected in blood. It might be a reliable biomarker on a group basis when measured by high-performance liquid chromatography, but due to large inter- and intra-individual variations it has little potential as a biomarker for individuals or a predictor of a specific disease (59).

8-hydroxy-2’-deoxyguanosine (8OhdG)

8-hydroxy-2’-deoxyguanosine (8OhdG) is a product of DNA oxidation damage caused by oxidative stress (60) and is used as a urinary biomarker of cancer risk. Traffic emissions also contribute to the increased levels of 8OhdG.

Reactive oxygen species (ROS)

Reactive oxygen species promote the destruction of endogenous antioxidants, thus reducing cellular antioxidant defences and damaging cellular lipids, proteins, or DNA (61).

Study type

RCTs

Of the 2 RCTs, one reported on change in LDL levels after smokers of at least 15 cigarettes per day were randomised to ad libitum use of cartridge vaping product with 16mg/mL or 0mg/mL nicotine e-liquid or to continue smoking for 4 weeks (11). Adjusted regression analysis found no statistically significant difference in LDL levels between the 3 RCT groups at 4-week follow-up (11).

Cross-over studies

Of the 11 cross-over studies, 6 reported on oxidative stress markers (13, 14, 16, 18, 20, 21).

Moheimani and others reported on LDL and HDL changes after non-users of tobacco and nicotine products were exposed to 60 puffs of a cartridge vaping product with 12mg/mL nicotine e-liquid, with 0mg/mL nicotine e-liquid and to sham vaping without e-liquid (20). Study authors reported no statistically significant changes in the LDL and HDL levels between nicotine vaping, non-nicotine vaping and sham vaping conditions.

Four cross-over studies compared changes in 8-isoprostane levels after a single use session (18, 21), 48 hours (14) and 5 days (13) of vaping, dual use, smoking or non-use conditions. One study found statistically significant increases in 8-isoprostane levels after a single use session of a cartridge vaping product with 16mg/mL nicotine e-liquid, smoking a cigarette and using an HTP (21). The study also reported statistically significant differences between all 3 conditions—with HTP use increasing 8-isoprostane levels least and smoking increasing 8-isoprostane levels most. The other 3 studies found no statistically significant differences in 8-isoprostane levels between different study conditions. Mastrangeli and others (18) conducted further inferential analysis which identified that having a longer smoking history was the strongest predictor of higher 8-isoprostane levels among participants. Benowitz and others argued that the 48-hour abstinence condition could have been too short for urinary 8-isoprostane levels to change (14), because it might take more than 3 days after smoking cessation to see the reduction in 8-isoprostane levels (62). Cobb and others reported that only 28% of 8-isoprostane

samples in exhaled breath condensate were above the lowest limit of detection (3.0 picograms per millilitre (pg/mL)), and no statistical comparisons could be made (13).

Biondi-Zoccai and others also measured changes in blood levels of soluble Nox2-derived peptide in smokers after single use of vaping product, smoking a cigarette or using an HTP (21). Findings were similar to the changes in 8-isoprostane levels reported from the same study—there was a statistically significant increase in soluble Nox2-derived peptide within all 3 groups, and levels increased most after smoking and least after using an HTP (21).

One study explored changes in MDA levels in smokers after vaping a tank type vaping product with 12mg/mL nicotine e-liquid or without nicotine for 7 minutes (16). No statistically significant changes in MDA levels were reported within and between groups after exposure to vaping product use (16).

Longitudinal studies

Five out of 6 non-randomised longitudinal studies reported on oxidative stress biomarkers (16, 24, 26, 27, 51). Walele and others reported on LDL and HDL changes at different follow-up points after smokers switched to ad libitum use of a cartridge vaping product with 16mg/mL nicotine e-liquid. The authors concluded that no ‘clear and consistent trends were observed’ in LDL and HDL changes during the 24-month follow-up period (24). Although some statistically significant changes in LDL and HDL levels were reported during the study period, interpretation of these changes is difficult because many participants continued to smoke (24).

One study reported on 8-isoprostane level changes in exhaled breath condensate of healthy smokers and smokers with asthma after they were exposed to 10 puffs of a cartridge vaping product with 10 to 15mg/mL nicotine e-liquid (51). The study authors reported a statistically significant 8-isoprostane increase after exposure to vaping product use in smokers with asthma and a non-significant increase in healthy smokers, although variability within these groups remained high and no difference in changes between the 2 groups was found (51).

Two studies conducted by the same research group from Greece reported on changes in MDA one (16) and 4 months (25) after smokers switched to ad libitum vaping product use. Both studies reported similar findings—a statistically significant decrease in MDA levels within vapers (16, 25) and ‘dual users’ (16) groups and no difference (16) or a statistically significant increase in MDA levels (25) within smokers’ groups. These results suggest that switching from smoking to vaping product use for at least one month might result in decreased MDA levels when compared with continued smoking.

An acute exposure study in 2 publications (26, 27) reported on statistically significant increase in blood serum ROS production after healthy non-smokers were exposed to 16 puffs of a disposable vaping product without nicotine. Authors also noted large

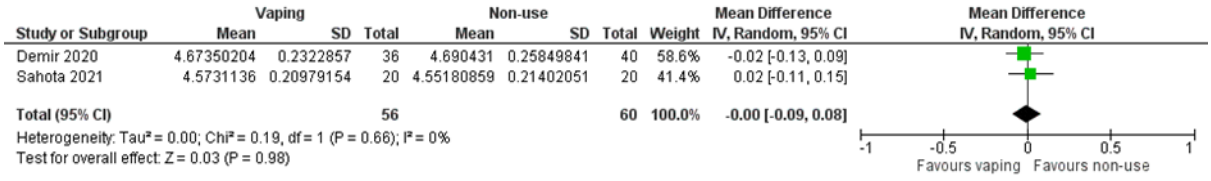
inter-subject variations in ROS production after vaping and associated it with individual differences in the response to oxidative challenge (27).

Cross-sectional studies

Eleven out of 24 cross-sectional studies reported on oxidative stress biomarkers (30, 32, 33, 35, 37, 41-44, 49, 51).

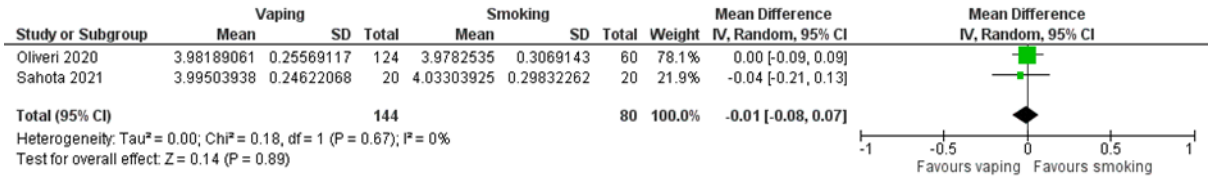
Five studies did not find statistically significant differences in blood LDL levels between self-reported vaping product users, smokers or non-users' groups (32, 33, 37, 41, 49). We meta-analysed results from 2 cross-sectional studies on LDL levels between vapers and non-users (figure 1). Pooled across the 2 studies, there were no statistically significant differences in average LDL levels between vapers and non-users (LMD: 0.00, 95% CI: -0.09, 0.08; 116 participants), with LDL levels ranging from 9% lower to 8% higher among vapers compared with non-users (GMR: 1.00, 95% CI: 0.91, 1.08). Heterogeneity between the 2 studies was low (I^2: 0%).

Figure 1. Meta-analysis of cross-sectional studies reporting on blood LDL levels between vapers and non-users



Findings differed across 7 cross-sectional studies that compared blood HDL levels between vaping product users, 'dual users', smokers or non-users (30, 32, 33, 35, 37, 41, 49). Following the algorithm for selecting studies for meta-analysis (methods: table 6), we pooled data from 2 cross-sectional studies comparing blood HDL levels between vaping product users and smokers (35, 41). The pooled data showed no statistically significant differences in average HDL levels between vapers and smokers (LMD: -0.01, 95% CI: -0.08, 0.07; 224 participants; figure 2), with average HDL levels ranging from 8% lower to 7% higher among vapers compared with smokers (GMR: 0.99, 95% CI: 0.92, 1.07). Heterogeneity between the 2 studies was low (I^2: 0%).

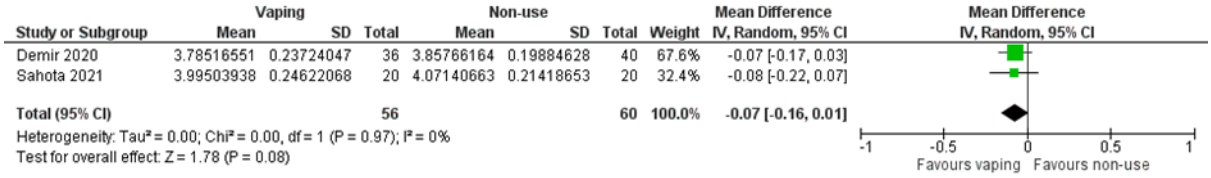
Figure 2. Meta-analysis of cross-sectional studies reporting on blood HDL levels between vapers and smokers



Two other cross-sectional studies also reported no differences in HDL levels between vaping product users, smokers and non-users (49) and between vaping product users and non-users (37). However, sample sizes of all 4 studies that did not find differences in HDL levels were relatively low, and across studies with larger sample sizes, HDL levels were higher among non-users compared with tobacco or vaping product users. For instance, Majid and others study (33), which recruited 530 participants, reported statistically significantly higher HDL levels among non-users compared with vaping product users, dual users and smokers. Two studies with largest sample sizes used the same KNHANES data including only men (30, 32) and reported higher HDL levels in non-users compared with dual users (30), or vaping product users and smokers (32).

Pooled data from 2 cross-sectional studies comparing average blood HDL levels between vaping product users and non-users (35, 41) showed no statistically significant differences, although the direction of results indicated lower HDL levels among vaping product users (LMD: -0.07, 95% CI: -0.16, 0.01; 116 participants; figure 3), with average HDL levels ranging from 15% lower to 1% higher among vapers compared with non-users (GMR: 0.93, 95% CI: 0.85, 1.01). Heterogeneity between the 2 studies was low (I^2=0%).

Figure 3. Meta-analysis of cross-sectional studies reporting on blood HDL levels between vapers and non-users



Four cross-sectional studies compared 8-isoprostane levels between vapers, smokers and non-users (31, 35, 43, 44). Two studies reported higher 8-isoprostane levels in smokers compared with vapers' groups (31, 35). Sakamaki-Ching and others did not find statistically significant differences in urinary 8-isoprostane levels between vapers and smokers but reported that 8-isoprostane levels were significantly higher among vapers and smokers compared with levels in non-users (43). Singh and others also reported 22% higher 8-isoprostane levels in exhaled breath condensate of vapers than non-users, but this difference was not statistically significant (44). In addition, Sakamaki-Ching and others reported that 8-isoprostane levels were statistically significantly elevated among participants older than 40-years of age and among women compared with men, suggesting that age and gender might be associated with higher sensitivity to oxidative stress (43).

Singh and others compared blood levels of MDA between self-reported exclusive vapers and non-users and reported no statistically significant differences (44).

Two cross-sectional studies studied differences in oxidative DNA damage biomarker 8OHdG levels—one recruited vapers, smokers and non-users (43) and another recruited participants with a diagnosis of periodontitis (42). Sakamaki-Ching and others found similar 8OHdG levels among vapers and smokers but statistically significantly higher levels among vapers than non-users (43); similar to 8-isoprostane levels, 8oHdG levels were higher among participants older than 40 years of age than younger participants. However, Karaaslan and others found no differences in 8OHdG levels between vapers, smokers and non-users and suggested that existing periodontal inflammation among participants might mask changes due to vaping or smoking (42).

Summary of studies reporting on oxidative stress biomarkers

One RCT, 6 cross-over studies, 5 non-randomised longitudinal studies, and 11 cross-sectional studies assessed oxidative stress biomarkers, specifically LDL, HDL, 8-isoprostane, soluble NOX2-derived peptide, MDA, 8OhdG and ROS. Most studies reported on differences in LDL, HDL and 8-isoprostane levels between vaping product users, smokers and non-users.

Evidence on blood LDL levels was consistent across studies with different designs indicating no differences after acute and short-to-medium use of vaping products, smoking or non-use of tobacco and nicotine products. Blood HDL levels were similar between vaping product users, smokers and non-users in studies with smaller sample sizes but were significantly higher among non-users in studies with larger sample sizes. Considering LDL and HDL associations with diet, physical activity and genetics, the current evidence does not indicate how vaping product use might affect LDL and HDL levels.

Comparisons of 8-isoprostane levels between vaping product, ‘dual use’, smoking and non-use groups after acute vaping or smoking exposures found mixed results. A few studies suggested that participants’ longer past smoking history, older age and female gender might be associated with elevated 8-isoprostane levels. In general, evidence from the included studies did not suggest strong associations between vaping and 8-isoprostane levels.

Evidence on vaping association with MDA levels came from one research group in Greece which reported no changes in MDA levels after acute vaping product use but detected significant reduction in MDA levels after smokers switched to vaping for one and 4 months. These findings need to be further confirmed.

Evidence on other oxidative stress biomarkers—soluble Nox2 derived peptide, 8OhdG and reactive oxygen species—was limited, mixed and likely confounded by other factors, therefore further conclusions about vaping associations with these biomarkers cannot be made.

Inflammation

The included studies assessed the association between vaping and all the inflammation biomarkers that we identified based on the US FDA sponsored workshop on biomarkers of potential harm (56).

Inflammation biomarkers

White blood cell (WBC) count

White blood cell count is a marker of systemic inflammation, and its increase is dose-dependent and positively associated with tobacco exposure. There are different WBC types, including lymphocytes, macrophages, neutrophils and eosinophils.

C-reactive protein (CRP)

An acute-phase, non-specific, marker of systemic and vascular inflammation detected in blood (56).

Interleukin-6 (IL-6)

A pro-inflammatory cytokine upstream of C-reactive protein (56), which is involved in inflammation and infection responses including the regulation of metabolic, regenerative and neural processes (63).

Interleukin-8 (IL-8)

Interleukin-8 is a chemoattractant cytokine produced by multiple tissue and blood cells in response to inflammation which specifically attracts and activates neutrophils in inflammatory regions (64).

Tumour necrosis factor alpha (TNF-α)

Tumour necrosis factor alpha is a proinflammatory cytokine involved in the acute phase reaction and implicated in many human diseases.

Soluble intercellular adhesion molecule 1 (sICAM-1)

Soluble intercellular adhesion molecule 1 is expressed in response to injury or inflammation of the endothelia.

Fibrinogen

Fibrinogen is an inflammation marker and a protein formed in response to vascular injures and infections (65).

Prostaglandin E2 metabolite (PGE-M)

Tobacco smoking is associated with elevated PGE2 levels which contributes to the development and progression of a number of cancers (56, 66).

Monocyte chemoattractant protein-1 (MCP)

Elevated levels of monocyte chemoattractant protein-1 in blood are associated with hypertension and increased cardiovascular diseases risk.

Study type

RCTs

One RCT reported on changes in various WBC counts in non-users who were asked to use a tank vaping product with 50%/50% propylene glycol/vegetable glycerine (PG/VG) ratio e-liquid without flavouring and nicotine at least twice a day for 4 weeks (12). No statistically significant changes were reported in total WBC count and in counts of macrophages, lymphocytes, neutrophils and eosinophils within vaping product users and non-users' groups at 4-week follow-up.

The same RCT also reported further evidence that vaping PG/VG e-liquid without flavouring and nicotine does not induce inflammation measured by IL-6, IL-8 and TNF-α levels. All these inflammation markers did not change statistically significantly after 4 weeks of daily vaping product use and did not differ from the non-users' group at the last study follow-up (12).

The other RCT reported on high-sensitivity CRP changes in smokers of at least 15 cigarettes per day who for 4 weeks switched to using a cartridge vaping product with 16mg/mL nicotine e-liquid, the same vaping product with 0mg/mL nicotine e-liquid or continued to smoke (11). The RCT did not find significant changes in CRP levels within or between study arms at 4 weeks follow-up (11).

Cross-over studies

Three out of 11 cross-over studies reported on vaping associations with inflammation markers, including changes in IL-6 and IL-8 (14), sICAM-1 (17) and fibrinogen (20).

Benowitz and others reported that, compared with 48-hour abstinence condition, participants' blood plasma IL-6 and IL-8 levels were statistically significantly higher after ad libitum vaping or smoking conditions, with no difference between the latter 2 conditions (14). The authors also reported no differences in IL-8 levels between users of different types of vaping product and found statistically significantly higher blood IL-6 levels in participants who used a modular versus cartridge type vaping product.

Kerr and others explored changes in healthy male smokers' sICAM-1 levels after acute exposure to 15 puffs on a tank type vaping product with 18mg/mL nicotine e-liquid and

smoking one cigarette (17)—no statistically significant changes were found in blood sICAM-1 levels within and between study conditions.

Moheimani and others explored changes in fibrinogen levels of non-users who were exposed to 60 puffs of a cartridge vaping product with 12mg/mL or 0mg/mL nicotine e-liquid or to sham vaping (without e-liquid) of the same vaping product device (20). The study authors reported no statistically significant differences in fibrinogen levels within and between study conditions after acute exposure sessions (20).

Longitudinal studies

Three out of 6 non-randomised longitudinal studies reported on associations between vaping and inflammation biomarkers (24, 26, 27, 51).

A longitudinal study of 24 months found no statistically significant changes in WBC counts at all follow-up points compared with baseline after smokers switched to ad libitum use of a cartridge vaping product with 16mg/mL nicotine (24). However, adherence to vaping product use was not enforced and many study participants continued smoking.

Chatterjee and others (27) explored changes in blood serum CRP and sICAM-1 levels after healthy non-smokers were exposed to 16 puffs of a disposable vaping product with 70%/30% PG/VG ratio, tobacco-flavoured and 0mg/mL nicotine e-liquid. The study reported statistically significant increases in CRP and sICAM-1 levels after acute exposure to vaping product use without nicotine. The authors also noted a considerable variation in inflammation markers at baseline (due to age, sex, weight, lipid levels, blood pressure, fitness, and antioxidant status) and concluded that the acute phase CRP increase by 20% to 25% after vaping product use was comparable to inflammatory disorders (27).

Kotoulas and others (51) compared multiple inflammation markers in exhaled breath condensate between healthy smokers and smokers with asthma 15 and 30 minutes after they were exposed to 10 puffs of a cartridge vaping product with medium nicotine content (exact nicotine levels were not reported). The study found no changes in IL-6, IL-8 and TNF-α levels between the 2 groups after exposure, and only TNF-α levels increased statistically significantly after vaping product use in smokers with asthma (51). Nevertheless, the study authors concluded that vaping product use altered airway inflammation in smokers with asthma more than in healthy smokers based on changes in a few other inflammation markers.

Cross-sectional studies

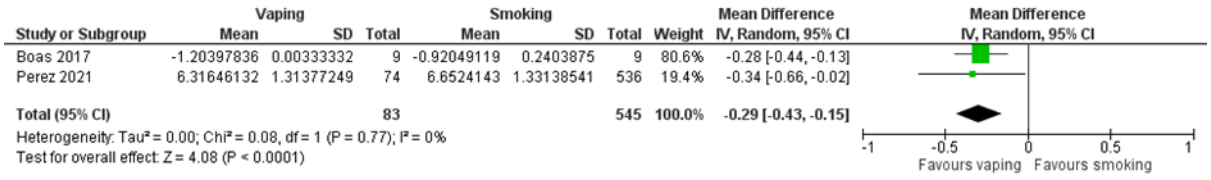
Seventeen out of 24 cross-sectional studies reported on inflammation markers (31, 32, 35, 36, 38-42, 44-50, 52, 53).

Six cross-sectional studies reported on all or some types of WBC counts (32, 35, 38, 41, 46, 50); here we discuss only studies that measured total WBC counts. Three studies that

reported on WBC levels, did not find statistically significant differences between smokers and vapers. A study with the highest sample size (n=1,208), which included only men, found no difference in WBC counts between self-reported vapers and smokers but reported statistically significantly lower WBC counts in non-users (32). Oliveri and others (35) found a 9% lower WBC count among vaping product users than smokers, but this difference was not statistically significant. Sahota and others (41) reported no differences in WBC counts between vapers, smokers and non-users.

Five cross-sectional studies reported on vaping associations with CRP levels (31, 32, 39, 44, 49), and we pooled data from 2 studies (31, 49) comparing vapers and smokers' CRP levels for meta-analysis (figure 4). Pooled data showed statistically significantly lower average blood CRP levels in vapers compared with smokers (LMD: -0.29, 95% CI: -0.43, -0.15; 628 participants); the geometric mean CRP levels were approximately 25% lower among vapers than among smokers (GMR: 0.75, 95% CI: 0.65, 0.86) and heterogeneity between the 2 studies was low (I^2: 0%).

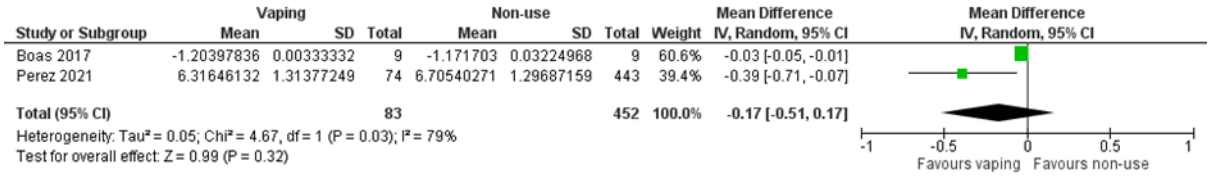
Figure 4. Meta-analysis of cross-sectional studies reporting on blood CRP levels between vapers and smokers



Two other studies reported similar blood (32) and salivary CRP (39) levels between users of vaping products and smokers, with large variance within study groups.

Regarding CRP differences between vaping products users and non-users, we again pooled data from 2 studies (31, 49). No statistically significant difference was found between average blood CRP levels in vapers and non-users (LMD: -0.17, 95% CI: -0.51, 0.17; 535 participants), with on average 40% lower to 19% higher blood CRP levels among vapers compared with non-users (GMR: 0.84, 95% CI: 0.60, 1.19). Heterogeneity between the 2 studies was substantial (I^2: 79%). The other 3 cross-sectional studies also did not find statistically significant differences in CRP levels between vapers and non-users (32, 39, 44).

Figure 5. Meta-analysis of cross-sectional studies reporting on blood CRP levels between vapers and non-users



Eight cross-sectional studies reported on IL-6 and/or IL-8 levels associated with vaping (31, 36, 38-40, 42, 44, 52). The methods of assessing IL-6 and IL-8 levels and the findings differed across the included studies. A study with the largest sample size (n=1,857) reported on blood plasma IL-6 levels among women between 18 and 49 years old who were self-reported vaping product users, smokers or non-users of tobacco and nicotine products (31); the study found no statistically significant differences in IL-6 levels between the 3 groups. Two other studies also reported no statistically significant differences in salivary IL-6 and IL-8 levels between college students (age range 18 to 25) who self-reported using vaping products in the past 30 days and those who did not use vaping products (40), and between vapers, 'dual users', smokers and non-users (39). On the other hand, one study reported statistically significantly higher IL-6 levels in gingival crevicular fluid of smokers compared with vapers (36), one study reported higher blood plasma IL-6 and IL-8 levels among exclusive users of vaping products compared with non-users (44), and one study used bronchoalveolar lavage and found statistically significantly higher IL-6 levels in self-reported vapers compared with non-users and no difference in IL-8 levels between vapers, smokers and non-users (38).

Two cross-sectional studies recruited participants with at least one dental implant (52) or periodontitis diagnosis (42). AlQahtani and others (52) reported statistically significantly higher IL-6 levels in the peri-implant sulcular fluid of 'dual users' compared with non-users of nicotine and tobacco products, and Karaaslan and others (42) found statistically significantly higher IL-8 levels in gingival crevicular fluid of vapers than smokers, but lower than in non-users' group.

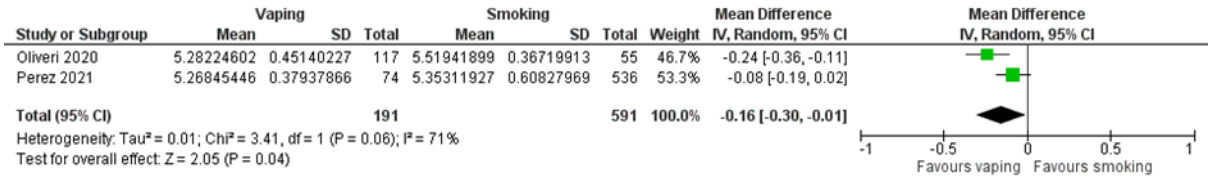
Seven cross-sectional studies reported on TNF-α levels measured in different biosamples (36, 38-40, 42, 52, 53). Similar to IL-6 and IL-8 findings, there were no consistent direction in results pertaining to TNF-α levels. Ashford and others (40) found no difference in TNF-α levels between vapers and non-vapers' groups, Song and others (38) also reported no difference between vapers, smokers and non-users' groups, Faridoun and others (39) found statistically significantly lower levels in non-users' group compared with vapers, 'dual users' and smokers' groups, while BinShabaib and others (36) reported statistically significantly higher TNF-α levels in smokers than vapers and non-users' groups.

Three studies compared TNF-α levels in participants with at least one dental implant (52, 53) or a diagnosis of periodontitis (42). The studies reported statistically significantly higher

TNF-α levels in smokers than vapers (42), 'dual users' than non-users (52) and no difference between users of vaping products and non-users (53).

Three cross-sectional studies reported on vaping associations with blood sICAM-1 levels (31, 35, 44). We pooled data from 2 studies (31, 35), which showed statistically significantly lower average blood sICAM-1 levels in vapers than smokers (LMD: 0.16, 95% CI: -0.30, -0.01; 782 participants; figure 6)—on average blood sICAM-1 levels were 15% lower among vapers compared with smokers (GMR: 0.85, 95% CI: 0.74, 0.99). Heterogeneity between the 2 studies was substantial (I^2: 71%).

Figure 6. Meta-analysis of cross-sectional studies reporting on blood sICAM-1 levels between vapers and smokers



Regarding blood sICAM-1 level differences between vapers and non-users, Perez and others study (31), which had the largest by sample size (n=1,857), did not find differences between the 2 groups, while the Singh and others study (44) reported statistically significantly higher levels among vapers than non-users (n=48).

Three studies compared blood fibrinogen levels between vapers, smokers and non-users (31, 44, 49) and all 3 found no statistically significant differences compared with the comparison groups.

Two studies reported on salivary PGE-M levels (44, 45). Both studies found no statistically significant differences in PGE-M levels between vaping product users and non-users, while Ye and others (45) also reported that PGE-M levels were statistically significantly elevated in smokers compared with vapers, 'dual users' and non-users.

One study compared blood plasma differences in MCP-1 levels between exclusive vapers and non-users and found no statistically significant differences between the 2 groups (44).

Summary of studies reporting on inflammation markers

Two RCTs, 3 cross-over studies, 3 non-randomised longitudinal studies and 17 cross-sectional studies assessed inflammation biomarkers, specifically WBC count, CRP, IL-6, IL-8, TNF-α, sICAM-1, fibrinogen, PGE-M and MCP-1. However, heterogeneity of the study designs, vaping and smoking definitions and methods for measuring biomarker levels preclude drawing clear conclusions about how vaping product use might compare to smoking or non-use in terms of inflammation.

Evidence from one RCT suggested that levels of IL-6, IL-8, TNF-α and WBC do not change after non-users vaped PG/VG e-liquid without nicotine, and a longitudinal study did not find changes in WBC count 24 months after smokers switched to vaping product use. Evidence from other studies regarding IL-6, IL-8 and TNF-α after exposure to vaping products with nicotine were mixed.

The other RCT found no significant differences in high-sensitivity CRP levels within or between groups 4 weeks after smokers switched to vaping product use with nicotine, vaping product use without nicotine or continued smoking. These findings, however, were not confirmed by other interventional or cross-sectional studies. Three cross-sectional studies were eligible for meta-analyses and showed lower blood CRP and sICAM-1 levels among vapers than smokers; levels of these inflammation markers were similar between vapers and non-users.

Endothelial function

Based on the endothelial function biomarkers that we had identified in accordance with the US FDA sponsored workshop on biomarkers of potential harm (56), none of the included studies measured von Willebrand factor or endothelial progenitor cells. Other endothelial function biomarkers that were reported in the included studies were as follows.

Endothelial function biomarkers

Flow-mediated dilation (FMD)

A marker of endothelial function showing a percentage change in arterial diameter increase. Every 1% improvement in flow-mediated dilation of brachial artery reduces the relative risk of cardiovascular events by 13% (67).

E-selectin and P-selectin

Both E-selectin and P-selectin are vascular adhesion molecules that mediate the adhesion of white blood cells to activated vascular endothelium. Smoking increases levels of these adhesion molecules.

Nitric oxide

Nitric oxide bioavailability is related with better vasodilation and increased blood flow, while oxidants reduce nitric oxide and impairs endothelial function.

Microvesicles (microparticles, extracellular vesicles)

Microvesicles are involved in intercellular communication and in the homeostatic regulation. There are different types of microvesicles (for example, endothelial, platelet, leukocyte and red blood) with the majority in the blood of healthy individuals consisting of platelet-derived microvesicles (68).

Study type

RCTs

George and others (11) measured changes in brachial artery flow-mediated dilation after smokers switched to ad libitum nicotine (16mg/mL using a cartridge vaping product) and non-nicotine vaping for 4 weeks. The trial found a statistically significant 1.5% improvement in FMD 4 weeks after switching from smoking to vaping with or without nicotine compared with participants who continued to smoke, despite their observation that around half of vaping product users were likely also smoking at the last follow-up (11). The improvement in vascular function was higher among female than male participants (11).

Cross-over studies

Findings of 4 cross-over studies that measured FMD changes after acute exposure to vaping products or smoking a tobacco cigarette differed (15, 18, 21, 23). Biondi-Zoccai and others (21) reported statistically significant decreases in FMD after smokers used 9 puffs of a cartridge vaping product with 16mg/mL nicotine e-liquid, smoked a cigarette or used an HTP tobacco stick—there was no difference in changes between vaping product use and smoking. Cossio and others (23), on the other hand, found no statistically significant FMD changes in tobacco naïve participants after they were exposed to 18 puffs of non-nicotine and 54mg/mL nicotine e-liquid vaping sessions. Haptonstall and others (15) reported no statistically significant changes in FMD after vapers used a cartridge (with 0 or 12mg/mL nicotine e-liquid) or a pod vaping product (50mg/mL nicotine salt e-liquid) for up to 60 puffs but found statistically significant decrease in FMD after a subgroup of smokers smoked a tobacco cigarette. Mastrangeli and others (18) reported that length of smoking history was independently associated with a greater decrease in FMD after acute exposure to both smoking and vaping product use.

Three cross-over studies measured E-selectin (17) and P-selectin levels (17, 19, 21) after acute vaping and smoking. No consistent findings were found between the studies. In Kerr and others study (17), smokers used 15 puffs of a tank vaping product with 18mg/mL and ad libitum smoked a cigarette—no differences were found in blood E-selectin levels within and between exposures, and levels of P-selectin statistically significantly decreased after vaping exposure but did not change after smoking. However, Biondi-Zoccai and others (21) reported statistically significant increase in P-selectin levels after vaping, smoking and HTP use, and Nocella and others (19) also reported statistically significant P-selectin level increases after both vaping and smoking sessions.

Two cross-over studies measured serum levels of nitric oxide bioavailability (18, 21). Biondi-Zoccai and others (21) reported a similar statistically significant decrease in nitric oxide bioavailability after acute vaping and smoking sessions, and Mastrangeli and others (18) indicated that the length of smoking history was independently associated with higher decrease in nitric oxide bioavailability after acute exposure to both smoking and vaping product use.

Mobarrez and others (22) reported on levels of endothelial and platelet derived microvesicles after occasional smokers were exposed to 30 puffs of a tank vaping product with 19mg/mL or 0mg/mL nicotine e-liquid. The study found statistically significant increases in both endothelial and platelet microvesicles 4 hours after exposure to a vaping product with nicotine e-liquid, while vaping of a non-nicotine e-liquid did not change the levels of microvesicles. The authors concluded that the observed stress on endothelial cells and platelets was associated with added nicotine in e-liquid (22).

Longitudinal studies

Two non-randomised longitudinal studies reported on FMD changes after non-smokers were exposed to 16 puffs of a disposable vaping product with non-nicotine e-liquid (26), and after smokers used 40 puffs of a tank type vaping product with 18mg/mL nicotine e-liquid (28). Both studies reported statistically significant reductions in FMD after exposure to vaping products.

Chatterjee and others found a statistically significant reduction in nitric oxide bioavailability in 80% of non-users who were exposed to 16 puffs of a disposable vaping product with non-nicotine e-liquid (27).

Staudt and others (29) reported statistically significant increase in blood plasma endothelial microvesicles 30 minutes after never smokers inhaled 10 puffs on a cartridge vaping product with nicotine and no differences in endothelial microvesicle levels after never smokers vaped non-nicotine e-liquid.

Cross-sectional studies

One cross-sectional study compared FMD levels between self-reported vapers, ‘dual users’, smokers and non-users and found no statistically significant differences between 4 study groups (34). To note, study measures were conducted after an 8 to 12 hours overnight fast from food and tobacco products.

Summary of studies reporting on endothelial function markers

One RCT, 4 cross-over studies, 3 non-randomised longitudinal studies, and one cross-sectional study assessed endothelial function biomarkers, specifically FMD, E-selectin and P-selectin, nitric oxide bioavailability and microvesicles. As for oxidative stress and inflammation markers, studies reported on multiple endothelial function markers but differed in study design, outcome measures and comparison groups, making it difficult to draw conclusions.

Many studies reported on changes in FMD after acute or short-to-medium exposure to vaping product use. The available evidence suggests that FMD tends to worsen (reduce) after acute exposure to vaping products with and without nicotine, but a single RCT found

that switching from smoking to vaping or even ‘dual use’ significantly improved (increased) FMD in a relatively short period of 4 weeks.

Evidence from 2 cross-over and one interventional study suggests that acute exposure to vaping might reduce the nitric oxide bioavailability similarly to acute smoking but also noted that past smoking history was an important confounder affecting the magnitude of change in nitric oxide bioavailability after acute exposure sessions.

Two studies reported statistically significant increase in blood endothelial microvesicles among occasional smokers and non-smokers after acute exposure to nicotine vaping, and no change in endothelial microvesicle levels was found after non-nicotine vaping.

Evidence was inconsistent or inconclusive for changes in E-selectin or P-selectin after acute exposure to vaping, and there were no studies exploring changes in these endothelial function markers or in microvesicles activation after longer exposure to vaping product use. In addition, only a single cross-sectional study included a non-user group in endothelial function comparisons, therefore no conclusions could be drawn about endothelial function differences between vapers and non-users of tobacco and nicotine products.

Other biomarkers

Platelet activation

Platelets, also called thrombocytes, are the smallest blood cells responsible for blood clotting. Platelet activation is a result of oxidative stress, and excessive and persistent platelet activation has been associated with the development of thrombosis, atherogenesis and angiogenesis (56). Activation of platelets has been associated with adverse cardiac events or mortality (69).

RCTs

Neither of the 2 included RCTs reported on vaping associations with platelet activation.

Cross-over studies

One cross-over study compared platelet aggregation in blood plasma at baseline and after smokers and non-users were exposed to 9 puffs of a cartridge vaping product with 16mg/mL nicotine e-liquid and after smoking a tobacco cigarette (19). At baseline, platelet aggregation did not differ between smokers and non-users, and it increased statistically significantly within both groups after vaping and smoking sessions. Statistically significantly higher increases in platelet aggregation were observed after smoking a cigarette in non-users than smokers, while the increase was similar between the 2 study groups after using the vaping product (19).

Longitudinal studies

A longitudinal study by Ikonomidis and others (25) examined the platelet function in smokers who for 4 months switched to ad libitum use of a tank type vaping product with 12mg/mL nicotine e-liquid. Based on platelet function measures using light transmission aggregometry and the novel Platelet Function Analyzer PFA-100, the study authors did not find statistically significant changes in the vapers' group but found a detrimental effect in the smokers' group at 4 months follow-up compared with baseline measures (25).

Cross-sectional studies

Two cross-sectional studies reported on platelet activation measures between self-reported vapers, smokers and non-users (37, 41). Neither of the studies found statistically significant differences in platelet activation between the comparison groups.

Summary of studies reporting on platelet function markers

We identified only 4 studies—one cross-over, one longitudinal and 2 cross-sectional—that assessed platelet activation measures, and no clear conclusions could be made on how acute or longer-term vaping might affect platelet function in comparison to smoking or non-use of tobacco and nicotine products.

8.5 Conclusions

This chapter examined findings from our systematic review on biomarkers of potential harm to health that are associated with oxidative stress, inflammation, endothelial function and platelet activation. These biomarkers are known to be associated with the development of multiple diseases (56) (methods: table 6). So, they are relevant to both our review questions—what effect does vaping have on biomarkers that are associated with the risk of cancer, respiratory, cardiovascular and other health conditions and what effect does vaping among people with existing health conditions have on disease outcomes. Several of the studies we included assessed biomarker changes in participants with existing health conditions (for example, asthma and dental diseases) but did not estimate how these changes affected outcomes of these health conditions. As these studies did not directly address the second review question, we presented their data alongside findings from participants from the general population.

Overall, we identified 41 unique studies reported in 43 publications, which reported biomarkers of potential harm associated with oxidative stress, inflammation, endothelial function and platelet activation biomarkers. There was significant methodological heterogeneity across the included studies, which likely resulted in discrepancies and variability of findings. First, studies assessed multiple biomarkers with different sensitivity, speed of onset or offset and reliability of predicting subsequent health risks—these differences obscured overall conclusions. Secondly, the studies used different definitions for vaping, smoking and non-use groups, usually did not bioverify smoking or vaping

status, and used varied methods (for example, different measures, biosamples and follow-up times) to compare a range of biomarkers between these groups. These differences precluded pooling data from more studies for meta-analyses and made comparisons between studies complicated. Furthermore, most included studies assessed acute vaping effects on oxidative stress, inflammation, endothelial and platelet functions, and because the explored biomarkers of potential harm mostly take weeks or months to normalise after people stop smoking, clear conclusions regarding longer-term vaping effects cannot be made. Finally, tobacco smoking (or vaping) is not the only known risk factor for detrimental changes in many of the explored biomarkers, and conclusions regarding vaping associations with the explored biomarkers are further limited by potential confounding of other variables and the lack of controlled studies. Therefore, findings need to be cautiously interpreted.

In line with our algorithm (methods: table 6), we carried out meta-analyses wherever possible, but a lack of consistency in study designs, biomarker reporting, group definitions and exposure periods resulted in few studies being included.

Oxidative stress

One RCT, 6 cross-over, 5 non-randomised longitudinal and 11 cross-sectional studies assessed oxidative stress biomarkers, specifically LDL, HDL, 8-isoprostane, soluble Nox2-derived peptide, MDA, 8OhdG and ROS.

No significant differences in LDL levels were found across studies between vapers, smokers and non-users' groups after acute and short-to-medium exposure. A meta-analysis of data from 2 cross-sectional studies also confirmed no difference in blood LDL levels between vapers and non-users.

Findings on HDL levels were inconsistent. Smaller studies reported no differences between vapers, smokers and non-users, and larger studies reported lower HDL levels among non-users compared with vapers and smokers. Two meta-analyses of cross-sectional studies found no difference in blood HDL levels between vapers compared with smokers or non-users.

Evidence for 8-isoprostane level changes after vaping product use was mixed. Studies emphasised longer past smoking history, older age and female gender as potential confounders for higher 8-isoprostane levels. In general, comparisons were limited by a lack of longer-term controlled exposure studies (considering time for biomarkers' levels to normalise) and potential confounding in non-randomised longitudinal and cross-sectional studies.

There was limited evidence for other oxidative stress biomarkers. The overall evidence from most of the included studies indicate no difference in vaping-associated oxidative stress risks in comparison with smoking or not using tobacco or nicotine products.

Inflammation

Two RCTs, 3 cross-over studies, 3 non-randomised longitudinal studies and 17 cross-sectional studies assessed inflammation biomarkers, specifically WBC count, CRP, IL-6, IL-8, TNF-α, sICAM-1, fibrinogen, PGE-M and MCP.

Pooled data from 3 cross-sectional studies indicated that average blood CRP levels were lower among vapers than smokers and similar between vapers and non-users, and that average blood sICAM-1 levels were significantly lower among vapers than smokers. However, controlled and longitudinal studies could not confirm these cross-sectional findings. Also, due to varied study designs and a lack of studies comparing the same outcome between the same study groups, no definite conclusions could be drawn on the association between vaping and any specific inflammation biomarker.

Endothelial function

One RCT, 4 cross-over studies, 3 non-randomised longitudinal studies, and one cross-sectional study assessed endothelial function biomarkers, specifically FMD, E-selectin and P-selectin, nitric oxide and microvesicles. No studies reporting on these biomarkers could be pooled for a meta-analysis.

While acute exposure studies showed similar short-term reductions in FMD parameters after vaping (with and without nicotine) and smoking sessions, a single RCT indicated that switching from smoking to vaping product use for 4 weeks significantly improved (increased) participants' FMD function.

Evidence from 2 cross-over studies and one interventional study indicated that acute vaping and smoking sessions led to similar reductions in nitric oxide bioavailability (more susceptibility to oxidative damage), but one study also noted that the reduction was directly associated with the length of past smoking history.

Evidence from one cross-over and one interventional study showed that acute nicotine vaping increased blood endothelial microvesicle levels while acute non-nicotine vaping did not change this outcome.

There was limited and inconsistent evidence regarding the other endothelial function biomarkers. Also, no conclusions could be made about the absolute effect of vaping on endothelial function as no controlled studies compared vapers and non-users.

Overall, acute vaping might induce endothelial dysfunction as much as acute smoking but switching from smoking to vaping product use might improve endothelial function in the longer-term.

Platelet biomarkers

Only one cross-over study, one longitudinal study and 2 cross-sectional studies assessed platelet activation measures. No data from these studies could be meta-analysed. So, evidence on the association between vaping and platelet function was limited, and no conclusions could be made regarding absolute effects of vaping on platelet activation or effects of vaping relative to smoking.

8.6 Implications

Considering the 2 human studies summarised by the NASEM report and the 41 studies (in 43 publications) included in our systematic review, research on effects that human vaping has on biomarkers that cut across diseases has grown in recent years, though is still at an early stage.

Our summary of the evidence on associations between vaping and oxidative stress, inflammation, endothelial function and platelet activation came from methodologically heterogeneous studies that mostly assessed acute exposure effects. These findings provide important insights allowing us to compare immediate effects between vaping and smoking. However, like smoking, it is the effects of long-term vaping that will be most relevant to public health, and the explored biomarkers of potential harm mostly take weeks or months to normalise after people stop smoking.

Our risk of bias assessments showed that most studies in this chapter had methodological concerns, and these should be addressed in future research as they limit interpretations of our findings. More research is needed, particularly in the UK, where we identified a lack of studies.

There is a need for future research among people who vape and have never smoked. This would allow us to determine long-term changes in biomarkers of potential harm exclusively due to vaping and not as a consequence of prior long-term smoking.

Furthermore, most biomarkers of potential harm are associated with multiple confounders not related with vaping or smoking (for example diet or physical activity). So, studies that explore acute effects of vaping and smoking, but do not include non-users as a comparison group, cannot clearly distinguish between the effects of vaping or smoking on these biomarkers. Due to these reasons, most studies that have been summarised in this chapter cannot inform us about the medium- or long-term vaping-associated risks via effects on the biomarkers we reviewed. This implies that further controlled studies with

adequate sample sizes, non-user comparison groups, and longer exposure and follow-up times are needed to clarify how switching from smoking to vaping affects the most reliable biomarkers of harm.

More research is also needed to develop ranges where biomarkers of potential harm become clinically relevant predictors of disease. This would improve the biomarkers' ability to estimate the pathways and contributions of vaping and smoking to multiple diseases.

Table 1. Summary of studies exploring vaping products (VP) use associations with biomarkers of potential harm associated with oxidative stress, inflammation, endothelial function and other health markers arranged by study design

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
RCT					
George et al., 2019, United Kingdom (11)	4 weeks (S-M)	n = 114 Smokers: self-reported smoking of ≥15 TC per day for ≥2 years. Mean age 46.8, 65.8% females.	4-week ad libitum use of: Vaping (n=37): cartridge VP (Vapourlites), 16 mg/mL nicotine. Compliance defined as CO<6ppm. Vaping, no nicotine (n=37): cartridge VP (Vapourlites), 0 mg/mL nicotine. Compliance defined as CO<6ppm. Smoking (n=40): own-brand TC.	Compliance at 4 weeks: 19 (51.4%) in vaping (VP) group had CO≥6 ppm. 19 (51.4%) in non-nicotine vaping (nnVP) group had CO≥6 ppm. Oxidative stress LDL: NS diff. within three arms at 4-week FU. Inflammation CRP: NS diff. within three arms at 4-week FU. Endothelial function FMD: Stat. sig. improvement in combined VP and nnVP arms compared with smoking (1.49%; 95% CI: 0.93-2.04; p<0.0001). Stat. sig. improvement in VP arm compared with smoking (1.44%,	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
				95% CI: 0.78-2.09; p<0.0001). Stat. sig. improvement in nnVP arm compared with smoking (1.52%, 95% CI: 0.90-2.15; p<0.0001). NS diff. between nnVP and VP arms (0.09%, 95% CI: -0.52-0.69; p=0.78).	
Song et al., 2020, US (12)	4 weeks (S-M)	n = 34 Non-users: self-reported healthy non-smokers, had smoked <100 TC in their lifetime and had not used a VP in the past year. Age range: 21-30.	4 weeks use at least twice per day. Vaping (n=14): 20 puffs over 60 minutes ≥2 times a day of a tank VP (Innokin iTaste) with 50%/50% PG/VG e-liquid without flavour or nicotine. Non-use (n=13): no use of VP.	Compliance at 4 weeks: self-reported and checked by the remaining e-liquid. Inflammation WBC: NS diff within VP (p=0.89) and non-users' group (p=0.99) at 4-week FU compared with baseline. NS diff. in change at FU between groups (p=0.51). Macrophages count: NS diff within VP (p=0.33) and non-users' group (p=0.95) at 4-week FU compared with baseline. NS diff. in change at FU between groups (p=0.51). Lymphocytes count: NS diff within VP (p=0.50) and non-users' group (p=0.46) at 4-week FU compared with baseline.	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
				NS diff. in change at FU between groups (p=0.45). Neutrophils count: NS diff within VP (p=0.77) and non-users' group (p=0.58) at 4-week FU compared with baseline. NS diff. in change at FU between groups (p=0.48). Eosinophils count: NS diff within VP (p=0.69) and non-users' group (p=0.63) at 4-week FU compared with baseline. NS diff. in change at FU between groups (p=0.79). IL-6: NS diff within VP (p=0.39) and non-users' group (p=0.57) at 4-week FU compared with baseline. NS diff. in change at FU between groups (p=0.68). IL-8: NS diff within VP (p=0.42) and non-users' group (p=0.74) at 4-week FU compared with baseline. NS diff. in change at FU between groups (p=0.97).	

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
				TNF-α: NS diff within VP (p=0.95) and non-users' group (p=0.36) at 4-week FU compared with baseline. NS diff. in change at FU between groups (p=0.58).	
Cross-over					
Benowitz et al., 2020, US (14)	48 hours (A)	n = 36 Dual users who used a VP ≥15 days and smoked ≥5 CPD over the past 30 days. A salivary cotinine level of ≥50 ng/mL. Mean (SD) age: 35.4 (11.7), 22% females, 61% mixed ethnicity, 14% white, 11% Latin, 8% Black, 6% Asian.	48-hour cross-over conditions in confinement: Vaping (n=36): ad lib use of own-brand VP (12 cartridge, 3 pod, 15 tank and 6 modular type) for 48 hours. Smoking (n=36): ad lib smoking of own-brand TC for 48 hours. Non-use (n=36): no use of tobacco or nicotine products for 48 hours.	Oxidative stress 8-isoprostane: NS diff. between groups. Inflammation IL-6: stat. sig. lower in non-use group compared with VP and smokers' groups (both p<0.01). NS diff. between VP and smoking group. IL-8: stat. sig. lower in non-use group compared with VP and smokers' groups (both p<0.01). NS diff. between VP and smoking group.	Some concerns
Biondi-Zoccai et al., 2019, Italy	Single use (A)	n=20 Smokers: healthy TC smokers with mean	Cross-over conditions separated by 1 week.	Oxidative stress 8-iso-prostaglandin F2α: stat. sig. increase within all groups after	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
(21)		(SD) smoking time in years: 15 (12). Mean (SD) age: 35 (13), 70% females, mean (SD) BMI: 24 (5).	Vaping (n=20): 9 puffs of cartridge VP (Blu pro) with tobacco flavoured 16 mg/mL nicotine strength e-liquid. Smoking (n=20): smoking a TC (Marlboro Gold). Others (n=20): using a single Amber label heets with HTP (IQOS).	exposure. Stat. sig. diff. between groups after exposure: smoking > VP group (p<0.001) > HTP group (p=0.004). Soluble Nox2-derived peptide: stat. sig. increase within all groups after exposure. Stat. sig. lower within VP group compared with smoking (p<0.001). Stat. sig. lower within HTP group compared with VP (p=0.004) and smoking (p=0.001) groups after exposure. Endothelial function FMD: stat. sig. decrease within all groups after exposure. Stat. sig. lower within smoking group compared with HTP (p=0.048) group after exposure. P-selectin: stat. sig. increase within all group after exposure. Stat. sig. higher in smoking group compared with VP and HTP groups (both p<0.001) after exposure.	

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
				Nitric oxide: stat. sig. decrease within VP (p=0.006) and smoking (p=0.006) group after exposure. NS diff. between groups after exposure.	
Cobb et al., 2020, US (13)	5 days (A)	n = 22 Dual users: self-reported smoking ≥10 TC per day for ≥1 year and using a VP ≥3 times per week for ≥3 months. Expired air CO ≥10 ppm and urinary cotinine of 3/6 of NicAlert test strip. Mean (SD) age: 41.9 (13.2), 50% females, 50% white, 45.5% African American, 4.5% Middle Eastern, 4.5% Hispanic.	5-day cross-over conditions: Vaping (n=22): ad lib use of own-brand cartridge VP with 2.4%-4.8% nicotine strength and menthol (81.8%) or tobacco (18.2%) flavoured e-liquid. Dual use (n=22): ad lib use of own-brand VP and TC. Smoking (n=22): ad lib use of own-brand TC with menthol (81.8%) or non-menthol (18.2%) flavour. Non-use (n=22): no TC or VP use for the last cross-over condition.	Missing data at FU: across all four conditions (n=264), 8.7% (n=23) samples were missing/unable to be analysed, 28% (n=74) samples were above the lowest limit of detection (3 pg/mL) and 63.3% (n=167) samples were below the LOD. Oxidative stress (n=18) 8-isoprostane: descriptively at day 5, mean levels were highest within dual use group > VP > smoking > non-use. Stat. sig. was not tested due to missing data.	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
Cossio et al., 2020, US (23)	Single use (A)	n = 16 Non-users: self-reported tobacco naïve participants who have not used nicotine products in the last 6 months. Mean (SD) age: 24 (3), 43.8% females, mean (SD) BMI: 23.2 (2.8).	Three cross-over conditions separated by ≥48 hours: Vaping (n=16): 18 4-second puffs every 20 seconds in 6 minutes on a cartridge type VP (White Cloud Cigarette) with menthol flavoured 5.4% nicotine strength e-liquid. Non-nicotine vaping (n=16): same use of the same VP with no nicotine e-liquid. Other (n=16): same use of a menthol cigarette-like pipe.	FU: 1 & 2 hours after exposure. Endothelial function FMD: NS diff. within all groups at all FUs.	Some concerns
Haptonstall et al., 2020, US (15)	Single use (A)	n = 136 Vapers (n=49): VP use for >1 year without smoking for >1 year, CO <10 ppm. Mean (SD) age: 27.4	Cross-over conditions separated by 4 weeks. Vaping (n=49): vaping a cartridge or pod VP (eGo-one, 1 Ω, or JUUL) for up to 60 puffs every	Endothelial function FMD: NS diff. after exposure within VP, nnVP or nicotine inhaler groups. Stat. sig. decrease within smokers' group after using a TC (p=0.02).	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		(5.5), 26.5% females, 59.2% Caucasian, 26.5% Hispanic, 10.2% Hawaiian, 2.1% African American. Smokers (n=40): Smoking for >1 year, CO >10 ppm. Mean (SD) age: 27.1 (5.5), 35% females, 62.5% Caucasian, 20% Asian, 12.5% African American, 5% Hispanic. Non-users (n=47): non-smokers or former smokers for >1 year, CO <10 ppm. Mean (SD) age: 26.3 (5.2), 53.2% females, 55.3% Caucasian, 19.1% Asian, 10.6% Hispanic, 8.5%	30 seconds with 1.2% nicotine strength strawberry flavour e-liquid (eGo-one) or 5% nicotine strength mint flavour salt (JUUL). Smoking (n=40, smokers): smoking own-brand TC in 7 minutes. Other (n=47, vapers): using nicotine inhaler with menthol flavour.		

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		African American.			
Ikonomidis et al., 2018, Greece (16)	Single use (A)	n = 70 Smokers attending hospital's smoking cessation unit. Mean (SD) age: 48 (5), 56% females.	Vaping (n=35): vaping for 7 minutes of a tank type VP (NOBACCO eGo Epsilon, 1100 mAh battery, 3.9 V) with 74.3%/20% PG/VG flavoured and 12 mg/mL nicotine strength e-liquid. Vaping, no nicotine (n=35): vaping for 7 minutes of the same VP with 0 mg/mL nicotine.	Oxidative stress MDA: NS diff. within all groups after acute exposure.	Serious risk of bias
Kerr et al., 2019, UK (17)	Single use (A)	n = 20 Smokers: smoking ≥1 TC per day. Mean (SD) age: 31.6 (10.5), all males, mean (SD) BMI: 25.7 (5).	Cross-over conditions separated by >24 hours. Vaping (n=20): 15 puffs on a tank type VP (1300mAh, 3.3 V battery voltage) with 66%/34% PG/VG ratio, 18 mg/mL nicotine strength and tobacco flavoured vaping liquid. Smoking (n=20): ad lib	Inflammation sICAM-1: NS diff. within and between groups after exposure. Endothelial function E-selectin: NS diff. within and between groups after exposure. P-selectin: stat. sig. decrease within VP group (p=0.026) after exposure compared with baseline, NS decrease within smoking group after exposure (p=0.117).	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
			smoking of a TC.		
Mastrangeli et al., 2018, Italy (18)	Single use (A)	n = 40 Self-reported smokers (n=20) and vapers (n=20). Participants' characteristics reported in tertiles based on differences in vitamin E; NR here.	Cross-over conditions separated by a week. Vaping (n=40): 9 puffs of a cartridge VP with 16 mg/mL nicotine strength e-liquid; use of approximately 0.6 mg of nicotine. Smoking (n=40): smoking a TC, approximately 0.6 mg nicotine.	Using generalised estimating equations, study explored independent predictors across all sample for the Endothelial function outcomes (adjusted for smoking status and cigarette type). Oxidative stress 8-iso-prostaglandin F2α: NS change after VP use and smoking compared with baseline (p=0.330). Stat. sig. higher in smokers compared with non-smokers (p<0.001). Endothelial function FMD (brachial): stat. sig. lower in smokers compared with non-smokers (p=0.020). Nitric oxide: stat. sig. lower in smokers compared with non-smokers (p<0.001).	Some concerns
Mobarrez et al., 2020, Sweden	Single use (A)	n = 17 Self-reported occasional smokers	Cross-over conditions separated by 1 week.	FUs: 2, 4 & 6 hours after exposure. Inflammation	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
(22)		of ≤10 TC per month. Mean (SD) age: 26 (3), 52.9% females, mean (SD) BMI: 24.4 (3).	Vaping (n=17): 30 3-second puffs every minute for 30 minutes of a tank VP (Joyetech eVic-VT, temperature 230 °C, 32 W, 0.2 Ω resistance) with 49.4%/44.4% PG/VG non-flavoured 19 mg/mL nicotine strength e-liquid. Vaping, no nicotine (n=17): same vaping regime of the same VP with 0 mg/mL nicotine e-liquid.	1) Endothelial cell derived: stat. sig. increase with peak at 4 hours FU within VP group (p<0.0001). NS diff. within nnVP group. 2) Platelet derived: stat. sig. increase with peak at 4 hours FU within VP group (p=0.0011). NS diff. within nnVP group. 3) Platelet derived extracellular vesicles & P-selectin: stat. sig. increase with peak at 4 hours FU within VP group (p=0.0018). NS diff. within nnVP group. 4) Platelet derived extracellular vesicles & CD40 L: stat. sig. increase with peak at 4 hours FU within VP group (p=0.001). Stat. sig. increase with peak at 6 hours within nnVP group (p=0.0434).	
Moheimani et al., 2017, US (20)	Single use (A)	n = 33 Self-reported non-users of VP or TC for ≥1 year. Mean (SD) age: 26.3 (0.9), 60.6%	Cross-over conditions separated by ≥4 weeks. Vaping (n=33): 60 3-second puffs with 30-seconds inter-puff	Oxidative stress LDL: NS diff. in change after exposure between groups (p=0.78). HDL: NS diff. in change after exposure between groups (p=0.30).	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		females, 45.5% white, 24.2% Asian, 15.1% black and 15.1% Hispanic.	intervals of a cartridge VP (Greensmoke or eGo One, 1.0 Ω) with tobacco (n=15) or strawberry (n=18) flavoured, 1.2% nicotine strength e-liquid. Vaping, no nicotine (n=33): vaping of the same VP with 0 mg/mL nicotine e-liquid. Sham vaping (n=33): vaping of the same VP without vaping e-liquid.	Inflammation Fibrinogen: NS diff. in change after exposure between groups (p=0.84).	
Nocella et al., 2018, Italy (19)	Single use (A)	n = 40 Healthy smokers (n=20) and non-users (n=20). Mean (SD) age: 28 (5.3), 52.5% females, mean (SD) BMI: 23.2 (2.9).	Cross-over conditions separated by a week. Vaping (n=40): both smokers and non-users used 9 puffs of a cartridge VP with 16 mg/mL nicotine and tobacco flavour. Smoking (n=40): both smokers and non-smokers smoked one TC	Endothelial function P selectin: stat. sig. higher at baseline in smokers compared with non-users' group (p<0.005) Stat. sig. increase after vaping & after smoking within non-users and smokers' groups (p<0.01 for both). Platelet activation (soluble CD40 ligand): stat. sig. higher at baseline in smokers compared with non-users' group (p<0.05). Stat. sig. increase after vaping &	Some concerns

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
			(0.6 mg nicotine).	after smoking within non-users and smokers' groups (p<0.01 for both). Other Platelet aggregation (%): NS diff. at baseline between non-users compared with smokers' group (p>0.05). Stat. sig. increase after vaping & after smoking within non-users and smokers' groups (p<0.01 for both). Stat. sig. higher increase in non-users compared with smokers after smoking (p<0.005). NS diff. in increase between non-users and smokers' group after VP use.	
Longitudinal					
Caporale et al., 2019, US & Chatterjee et al., 2021, US (26, 27)	Single use (A)	n = 31 Healthy self-reported non-smokers. Mean (SD) age: 24.3 (4.3), 45.2% females, 74.2% white, 16.1% Asian, 9.7% African Americans, mean	Vaping (n=31): 16 3-seconds puffs in 3 minutes of a disposable VP (ePuffer, 3.7 V battery, 2.7 Ω single coil) with 70%/30% PG/VG, tobacco flavoured and 0 mg/mL nicotine strength e-liquid.	Oxidative stress ROS: stat. sig. increase in all participants after vaping (p<0.0005). Inflammation CRP: stat. sig. increase in all subjects after vaping (p<0.0005). sICAM-1: stat. sig. increase in 90%	Low risk of bias

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		(SD) BMI: 22.9 (2.4).		of subjects after vaping (p<0.0005). Endothelial function FMD (superficial femoral artery): stat. sig. decreased after exposure (p<0.001). Nitric oxide: stat. sig. decrease in 80% of participants after vaping (p<0.0005).	
Ikonomidis et al., 2018, Greece (16)	1 month (S-M)	n = 70 Smokers attending hospital's smoking cessation unit. Additional group of smokers (n=20) was a control group for FU at 1 month. Mean (SD) age: 48 (5), 56% females.	Vaping (n=42): ad lib use of a VP with 12 mg/mL nicotine. Dual use (n=24): ad lib use of the VP and own-brand TC. Smoking (n=20): ad lib smoking of own-brand TC.	Compliance at 1 month FU Self-reported mean (SD) CPD: Vapers: 0; Dual users: 5 (4); Smokers: 24 (7.1). Oxidative stress MDA: Stat. sig. decrease within vapers (p=0.001) and dual users' (p=0.001) groups at 1 month FU compared with baseline. NS diff. within smokers' group (p=0.3) at 1 month FU.	Serious risk of bias
Ikonomidis et al., 2020, Greece (25)	4 months (S-M)	n = 40 Self-reported smokers of mean 25.9 CPD. Mean (SD) age 44.8 (11.3), 80% females.	4-month ad libitum use of: Vaping (n=20): tank VP (NOBACCO eGo Epsilon BDC), 4.5%	Compliance at 4 months: all VP group had eCO <10 ppm, 5/20 in VP group self-reported using 3-4 CPD. Oxidative stress MDA: Stat. sig. decrease in VP	Moderate risk of bias

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
			non-specified flavouring, 74.3% to 20% PG/VG ratio, 12 mg/mL nicotine Smoking: own-brand TC.	(p=0.03). Stat. sig. increase in smoking (p=0.03). Other Light Transmission Aggregometry - Epinephrine stimulator (LTA EPI): NS diff. in VP (p=0.263). Stat. sig. decrease in smoking (p<0.001). PFA: NS diff. in VP (p=0.454). Stat. sig. increase in smoking (p<0.047).	
Kuntic et al., 2020, Germany (28)	Single use (A)	n = 20 Healthy smokers smoking on average 14 CPD and having 11.6 pack-years. Mean (SD) age: 34.7 (10.2), 50% females, mean (SD) BMI: 26.8 (3.9).	Vaping (n=20): 40 puffs with 30-second inter-puff interval for 20 minutes of a tank VP (Joytech eGo C) with tobacco flavoured, 18 mg/mL nicotine strength e-liquid.	FU: 15 minutes after use. Endothelial function FMD: stat. sig. decrease 15 minutes after exposure (p=0.017) compared with baseline.	Moderate risk of bias
Staudt et al., 2018, US (29)	Single use (A)	n = 10 Never smokers: self-reported, validated by <2 ng/mL nicotine and <5 ng/mL	Vaping, nicotine (n=7): 10 puffs on a cartridge VP (Blu) followed by other 10 puffs after 30 minutes (nicotine content NR).	FU 30 minutes after exposure. Endothelial function Endothelial microvesicles (blood plasma): stat. sig. higher levels compared with baseline after	Low risk of bias

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		cotinine in urine. Mean (SD) age: 40.2 (9.7), 50% females, 70% Black, 30% Hispanic.	Vaping non-nicotine (n=3): same regime on the same VP without nicotine.	nicotine vaping (p<0.05). NS diff. compared with baseline after non-nicotine vaping (p>0.9).	
Walele et al., 2018, UK (24)	24 months (L)	n = 209 Smokers: self-reported smoking of 5-30 TC per day for ≥1 year. Mean (SD) age among those who switched (n=109): 38.7 (10.2), 44.1% females, mean (SD) BMI: 26.2 (4).	24 months ad lib use with FUs at 1, 3, 6, 12, 18 and 24 months: Vaping (n=209): cartridge VP (Puritane) with 1.6% nicotine strength, 67.5%/30% PG/VG vaping liquid with tobacco or menthol flavour.	Compliance: 102/209 (48.8%) followed-up at 24 months and were abstinent from smoking cigarettes for ≥80% of the study days. Oxidative stress LDL: Stat. sig. increase by 4% at month 6, by 5% at month 12 and by 4.7% at month 18 compared with baseline. NS diff. at other FU points. HDL: Stat sig. decline by 3.6% at month 12 compared with baseline. NS diff. at other FU points. Inflammation WBC: NS diff. at all FU points compared with baseline.	Moderate risk of bias
Cross-sectional					
Ashford et al., 2020, US (40)		n = 61 College students	Vapers (n=32): self-reported past 30 days VP use, 34.4% have used	Inflammation IL-6: NS diff. between vapers and non-vapers' group (p=0.56).	7/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		attending on-campus meetings. Age range: 18-25, 52.5% females, 95.1% white, 3.3% black, 1.6% Hispanic.	TC and 59.4% have used cannabis in the past month. Non-vapers (n=29): self-reported past 30 days non-use of VP, 10.3% have used TC and 17.2% have used cannabis in the past month.	IL-8: NS diff. between vapers and non-vapers' group (p=0.47). TNF-α: NS diff. between vapers and non-vapers' group (p=0.14).	
BinShabaib et. al., 2019, Saudi Arabia (36)		n = 135 Vapers (n=44): mean (SD) age: 46.5 (1.7), 4.5% females. Smokers (n=46): mean (SD) age: 44.2 (3.5), 6.5% females. Non-users (n=45): mean (SD) age: 40.6 (3.3), 13.3% females.	Vapers (n=44): self-reported VP users ≥1 per day. Smokers (n=46): self-reported smokers of >4 TC per day for >1 year. Non-users (n=45): self-reported never users of tobacco or nicotine products.	Inflammation IL-6: stat. sig. higher in smokers compared with vapers (p<0.05) and non-users' group. TNF-α: stat. sig. higher in smokers compared with vapers (p<0.05) and non-users' group.	7/20
Boas et al., 2017, US (49)		n = 31 VP users (n=11:	Self-reported: Vapers (n=11): VP use	Oxidative stress LDL: NS diff. between groups (p=0.91).	12/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		mean (SD) age: 29 (1.5), 2 females, 6 white, 1 African America, 1 Asian, 1 Hispanic. Smokers (n=10): mean (SD) age: 27.1 (1.6), 2 females, 7 white, 1 Asian, 1 Hispanic. Non-users (n=10): mean (SD) age: 28 (1.6), 3 females, 6 white, 2 Asian, 1 Hispanic.	most days for >1 year. Smokers (n=10): smoking for >1 year. Non-users (n=10): no use of VP or TC or had stopped smoking >1 year.	HDL: NS diff. between groups (p=0.62). Inflammation CRP: NS diff. between groups (p=0.32). Fibrinogen: NS diff. between groups (p=0.67).	
Demir et al., 2020, Turkey (37)		n = 76 Vapers (n=36): mean (SD) age: 41.7 (10.1), 22.2% females, mean (SD) BMI: 27.3 (5.8). Non-users (n=40): mean (SD) age: 39.1 (11.4), 25% females,	Vapers (n=36): self-reported VP use for ≥6 months. Non-users (n=40): self-reported no use of tobacco or nicotine products.	Oxidative stress LDL: NS diff. between groups (p=0.775). HDL: NS diff. between groups (p=0.216). Other Platelet activation: NS diff. between groups (p=0.344).	15/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		mean (SD) BMI: 26 (3.4).			
Faridoun et al., 2021, US (39)		n = 64 Mean (SD) age: 51.7 (16.8), 42% females, 64.1% Caucasian, 25% African American, 10.9% of other ethnicity. 10.9% had diabetes diagnosis.	Self-reported: Vapers (n=15) Dual users (n=16) Smokers (n=18) Non-users (n=15).	Inflammation CRP: NS diff. between groups (p=0.075), descriptively, smokers>vapers>dual users>non-users. IL-6: NS diff. between groups (p=0.901). IL-8: NS diff. between groups (p=0.99). TNF-α: stat. sig. lower in non-users group compared with other three groups (p=0.01).	6/20
Fetterman et al., 2020, US, (34)		n = 467 Vapers (n=36): mean (SD) age: 29 (6), 28% females. Dual users (n=52): mean (SD) age: 33 (7), 47% females. Smokers (n=285):	VP users (n=36): current vaping ≥5 days a week, no current smoking for >3 months Dual users (n=52): current vaping and smoking ≥5 days a week, smoked >100 TC in their lifetime	Results were adjusted for age, sex, race and study site. Endothelial function FMD: NS diff. between groups (p=0.68)	12/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		mean (SD) age: 32 (7), 42% females. Non-users (n=94): mean (SD) age: 29 (6), 56% females.	Smokers (n=285): current smoking ≥ 5 days a week, no current vaping Non-users (n=94): no current use of nicotine products, smoked <100 TC in their lifetime, urinary cotinine <10 ng/mL		
Ghosh et al., 2018 & Ghosh et al., 2019, US (47, 48)		n = 42 All participants underwent bronchoscopies. VP users (n=14): mean (SD) age: 26.1 (8.3), 28.6% females, mean (SD) BMI: 29.8 (6.6) kg/m^2. Smokers (n=14): mean (SD) age: 29.5 (5.6), 42.9% females, mean (SD) BMI: 27.8 (6.1) kg/m2.	Self-reported: VP users (n=14): former or never smoker, using a VP for 1-2.5 years. Smokers (n=14): self-reported TC use with mean (SD) 7.76 (5.6) pack-years. Non-users (n=14): self-reported never smokers.	Inflammation Macrophages count: NS diff. between groups (p>0.05). Lymphocytes count: NS diff. between groups (p>0.05). Eosinophils count: NS diff. between groups (p>0.05).	14/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		Non-users (n=14): mean (SD) age: 25.8 (7.3), 71.4% females, mean (SD) BMI: 26.2 (5.9) kg/m^2.			
Kim et al., 2020, South Korea (30)		n = 7,505 Vapers (n=62): mean age NR. Dual users (n=337): mean (SE) age: 36.7 (0.7). Smokers (n=4,079): mean (SE) age: 43.6 (0.3). Non-users (n=3,027): mean (SE) age: 39.8 (0.4)	Vapers (n=62): VP use for the past month and no smoking. Dual users (n=337): smoked >100 TC in lifetime, currently smoke and have used a VP in the past month. Smokers (n=4,079): smoked >100 TC in lifetime, currently smoke and have not used a VP in the past month. Non-users (n=3,027): smoked <100 TC in lifetime or never smoked and have not used a VP for the past month.	Oxidative stress HDL: stat. sig. lower in dual users compared with never smokers' group ($p<0.05$).	9/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
Majid et al., 2021, US (33)		n = 530 VP and DU characteristics provided by e-cigarette and pod use. NR here. Smokers (n=290): mean (SD) age: 33 (7), 44% females. Non-users (n=104): mean (SD) age: 29 (6), 51% females.	Vapers (n=65): self-reported use of a VP for ≥5 days per week and no use of TC for ≥3 months. Included e cigarette (n=42) and pod vapers (n=23). Dual users (n=66): self-reported use of VP and TC for ≥5 days per week and smoked >100 TC in their lifetime. Smokers (n=290): self-reported smoking for ≥5 days per week, smoked >100 TC in their lifetime, no current use of VP. Never users (n=104): self-reported no current use of TC or VP, smoked <100 TC in their lifetime, urinary cotinine levels <10 ng/mL. Dual users (n=66) included dual e-cigarette	Oxidative stress LDL: NS diff between never users compared with vapers (p=0.162), dual users (p=0.267) and smokers (p=0.739). HDL: stat. sig. higher in never users compared with vapers (p=0.049), dual users (p=0.002) and smokers (p=0.019).	12/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
			vapers (n=47) and dual pod vapers (n=19).		
Moon et al., 2020, South Korea (32)		n = 1208 All men. VP users (n=63): Mean (SD) age: 37.1 (11.5). Smokers (n=715): Mean (SD) age: 42.3 (11.3). Non-users (n=430): Mean (SD) age: 38.4 (13.3).	Vapers (n=63): self-reported VP use at least once in the last month. Smokers (n=715): self-reported smoking >100 TC in their lifetime and currently smoking 'sometimes' or 'everyday'. Non-users (n=430): self-reported non-users of TC and VP and not former smokers.	Measures after >8 hours overnight abstinence. Oxidative stress LDL: NS diff. between groups. HDL: stat. sig. higher in non-users compared with VP and smokers' groups (p<0.001). NS diff. between VP and smokers' groups. Inflammation WBC: stat. sig. lower in non-users compared with VP and smokers' groups (p<0.001). NS diff. between VP and smokers' groups. CRP: NS diff. between groups.	11/20
Olivieri et al., 2020, US (35)		n = 217 Vapers (n=132): mean (SD) age: 44.4 (8.3), 46.2% females, 54.5%	Vapers (n=132): former smokers of ≥10 TC per day for ≥10 years, currently using only a VP ≥6 months.	Results adjusted for age, gender, BMI group and race. Oxidative stress HDL: NS diff. between smokers and vapers' groups (p=0.54).	15/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		white, 30.3% African American, 15.2% of multiracial or other ethnicity. Smokers (n=62): mean (SD) age: 47.1 (8.5), 51.6% females, 77.4% white, 17.7% African American, 4.8% of other ethnicity.	Smokers (n=62): smoke ≥10 TC per day for ≥10 years and did not use other nicotine products in the past 30 days.	8-iso-prostaglandin F2α: stat. sig. higher levels in smokers' group compared with vapers' group (p=0.0194). Inflammation WBC: NS diff. between smokers and vapers' groups (p=0.0588). sICAM1: stat. sig. higher levels in smokers' group compared with vapers' group (p=0.0165).	
Perez et al., 2021, US (31)		n = 1857, women between ages 18-49. % within age groups for vapers/smokers/non-users: 18-24: 16.1%/16.4%/22.9%, 25-34: 36.5%/35.6%/27.5%, 35-49: 47.4%/48%/49.6%.	Vapers (n=74): self-reported VP use some or every day. Smokers (n=536): self-reported had smoked >100 TC, current some or everyday smoking. Non-users (n=443): self-reported never use of TC or VP.	Oxidative stress 8-iso-prostaglandin F2α: stat. sig. higher levels in smokers compared with vapers (p<0.03) and non-users' (p<0.04) group. Inflammation CRP: NS diff. between groups. IL-6: NS diff. between groups. sICAM1: stat. sig. higher in smokers compared with vapers (p<0.03) and non-users' (p<0.04) groups. NS diff. between vapers and non-	16/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
				users Fibrinogen: NS diff. between groups.	
Sahota et al., 2021, US (41)		n = 60 Vapers (n=20): mean (SD) age: 25.7 (4.2), 35% females, mean (SD) BMI: 26.1 (3). Smokers (n=20): mean (SD) age: 27 (3.1), 35% females, mean (SD) BMI: 24.5 (4.9). Non-users (n=20): mean (SD) age: 24.6 (1.9), 40% females, mean (SD) BMI: 23.1 (3.4).	Vapers (n=20): self-reported VP use for ≥3 months. Smokers (n=20): self-reported smoking ≥5 TC per day, had smoked >500 TC in their lifetime. Non-users (n=20): self-reported non-smokers.	Oxidative stress LDL: NS diff. between groups (p=0.95). HDL: NS diff. between groups (p=0.64). Inflammation WBC: NS diff. between groups (p=0.70). Lymphocytes count: NS diff. between groups (p=0.18). Neutrophiles count: NS diff. between groups (p=0.37). Eosinophil count: NS diff. between groups (p=0.93). Other Platelet activation: NS diff. between groups (p=0.54).	10/20
Sakamaki-Ching et al., 2020, US (43)		n = 53 Vapers (n=21): age range: 19-66, 10 females.	Vapers (n=21): abstained from smoking for ≥6 months, verified by NNAL levels lower than smokers'.	Oxidative stress 8OhdG: NS diff. between vapers and smokers' group (p=0.75). Stat. sig. higher in vapers' group compared with non-users' group	9/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		Smokers (n=13): age range: 24-75, 7 females. Non-users (n=19): age range: 23-66, 10 females.	Smokers (n=13): self-reported. Non-users (n=19): verified by cotinine ≤1 ng/mg.	(p=0.01). Diff. between smokers and non-users' group NR. 8-isoprostane: NS diff. between vapers and smokers' group (p=0.96). Stat. sig. higher in vapers' group compared with non-users' group (p=0.03). Diff. between smokers and non-users' group NR. Analysis further divided groups by gender and age, with a cut-off of 40 years old. 8-OHdG levels were elevated among those ≥41 years of age compared with <41 old participants. 8-isoprostane levels were elevated among those ≥41 years of age compared with <41 old participants and among women compared with men.	
Singh et al., 2019, US (44)		n=48 Healthy participants without chronic diseases or	Vapers (n=22): exclusive VP users. Non-users (n=26): never users of tobacco	Oxidative stress 8-isoprostane: NS increase by 22% in VP group compared with non-users.	8/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		respiratory infections. Mean age: 34.6, 56.3% females, 60.4% white, 18.8% African American, 14.6% Asian, 6.3% Hispanic, mean BMI: 26.1 kg/m^2.	products.	MDA: NS diff between groups. MCP-1: NS diff. between groups. Inflammation CRP: NS diff between groups. IL-6: stat. sig. higher in VP group compared with non-users ($p<0.05$). IL-8: stat. sig. higher in VP group compared with non-users ($p<0.05$). sICAM-1: stat. sig. higher in VP group compared with non-users ($p<0.05$). Fibrinogen: NS diff between groups. PGE-M: NS diff between groups.	
Song et al., 2020, US (38)		n = 73 Mean (range) age: 26 (21-30), 47% females.	Vapers (n=15): self-reported. Smokers (n=16): self-reported. Non-users (n=42): smoked <100 TC in their	Inflammation Macrophages count: stat. sig. higher in smokers compared with vapers' group ($p=0.02$). NS diff. between vapers and non-users' groups ($p=0.13$). Lymphocytes count: NS diff.	7/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
			lifetime and not used a VP for the past year.	between groups. Neutrophiles count: NS diff. between groups. Eosinophiles count: NS diff. between groups. IL-6: NS lower levels in vapers compared with smokers' group (p=0.07). Stat. sig. higher in vapers compared with non-users' group (p=0.02). IL-8: NS diff. between groups. TNF-α: NS diff. between groups.	
Tsai et al., 2019, US (46)		n = 43 Vapers (n=15): mean (range) age: 27 (21-30), 33% females, 80% white. Smokers (n=16): mean (range) age: 26 (21-30), 25% females, 88% white.	Self-reported: Vapers (n=15): non-smokers who used ≥1 mL vaping liquid for ≥3 months. Smokers (n=16): smoked ≥10 TC per day for ≥6 months and no VP use for ≥1 year.	Inflammation Macrophages count: NS diff. between vapers compared with smokers (p=0.06) and non-users' group (p=0.53). Stat. sig. higher levels in smokers compared with non-users' group (p=0.006). Lymphocytes count: NS diff. between all groups (p>0.05).	9/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		Non-users (n=12): mean (range) age: 26 (21-30), 58% females, 83% white.	Non-users (n=12): smoked <100 TC in their lifetime, did not smoke or use VP in the past year. Validated by salivary cotinine levels.	Neutrophiles count: NS diff. between all groups (p≥0.05).	
Ye et al., 2020, US (45)		n = 48 Vapers (n=12): mean (SD) age: 34.9 (11.5), 2 females, 8 white, 1 African American, 1 Asian and 2 of other ethnicities. Dual users (n=12): mean (SD) age: 39.4 (11.8), 5 females, 4 white, 4 African American, 2 Asian and 2 of other ethnicities. Smokers (n=12): mean (SD) age: 40.3 (16), 7 females, 7	Self-reported: Vapers (n=12) Dual users (n=12) Smokers (n=12) Non-users (n=12)	Inflammation PGE-M: NS diff. between vapers compared with non-users (p=0.9) and dual users (p=0.84). Stat. sig. lower in vapers compared with smokers' group (p=0.006). Stat. sig. higher in smokers compared with dual users (p=0.01) and non-users' (p=0.004) groups. NS diff. between dual users and non-users' groups (p=0.73).	12/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		white, 2 African American, 3 Asian. Non-users (n=12): mean (SD) age: 35.7 (12.5), 10 females, 5 white, 4 African American and 3 of other ethnicities.			
Participants with asthma					
Aboelnaga et al., 2018, Egypt (50)	Cross-sectional	n = 130 Participants diagnosed with asthma but without a respiratory infection or asthma exacerbation within the last 2 months. VP users (n=41): mean (SD) age: 30.4 (4.7), 53.7% females, mean (SD) BMI: 28.2 (6.5) kg/m^2. Smokers (n=41):	Self-reported: VP users (n=41): current VP use for ≥12 months. Smokers (n=41): current smokers having smoked >99 TC in lifetime. Non-users (n=48): definition NR.	Inflammation Eosinophils count: stat. sig. diff. between groups with non-users > VP & smokers groups (p=0.001). NS diff. between VP and smokers groups.	5/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
		mean (SD) age: 29.5 (5.3), 46.3% females, mean (SD) BMI: 27.1 (5.8) kg/m^2. Non-users (n=48): mean (SD) age: 30.3 (4.9), 54.2% females, mean (SD) BMI: 26.7 (6.8) kg/m^2.			
Kotoulas et al., 2020, Greece (51)	Longitudinal, single use (A)	n = 50 Smokers with asthma diagnosis (n=25), mean (SD) age: 40.6 (10.8), 48% females, mean (SD) BMI: 26 (5) kg/m^2 and healthy smokers (n=25), mean (SD) age: 39.9 (10.2), 68% females, mean (SD) BMI: 26.5 (3.8) kg/m^2).	Vaping (n=50): 10 puffs with 30 s inter-puff intervals for 5 minutes on a cartridge VP (NOBACCO, 1.2 Ω coil resistance) using 1 to 1.5 mL e-liquid of medium nicotine content.	FU: 30 min, 60 min after VP use. Oxidative stress 8-isoprostane: stat. sig. increase within smokers with asthma (p=0.008) and NS increase within healthy smokers (p=0.53). NS diff. in change between groups (p=0.683). Inflammation IL-6: NS diff. within groups after exposure. NS diff. between groups after exposure (p=0.239).	Low risk of bias

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
				IL-8: NS diff. within groups after exposure. NS diff. between groups after exposure (p=0.091). TNF-α: stat. sig. increase within smokers with asthma (p=0.028) and NS change within healthy smokers (p=0.737). NS diff. between groups after exposure (p=0.241).	
Participants with dental diagnoses					
Al-Aali et al., 2018, Saudi Arabia (53)	Cross-sectional	n = 92 All male participants with ≥1 dental implant for ≥36 months. Vapers (n=47): mean (SD) age: 35.8 (6.2). Non-users (n=45): mean (SD) age: 42.6 (2.7).	Self-reported: Vapers (n=47): current use of a VP for the past year. Non-users (n=45): self-reported non-smokers who had never used a VP.	Inflammation TNF-α: NS diff. between groups (p<0.05).	8/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
AlQahtani et al., 2018, Saudi Arabia (52)	Cross-sectional	n = 160 Participants with ≥1 dental implant in service for≥3 years.	Self-reported: Dual use (n=40): vaping and smoking 6.5 (0.9) CPD. Smokers (n=40): smoking 14.6 (3.8) CPD. Non-users (n=40): non-use of tobacco and nicotine products. Other (n=40): waterpipe use and smoking 5.9 (1.1) CPD.	Inflammation IL-6: stat. sig. higher in dual users compared with non-users' group (p<0.05). TNF-α: stat. sig. higher in dual users compared with non-users' group (p<0.01).	7/20
Karaaslan et al., 2020, Turkey (42)	Cross-sectional	n = 57 Participants were diagnosed as having periodontitis diagnosis. Mean (SD) age: 35.2 (2.2), 31.6% females.	Self-reported: Vapers (n=19): former smokers who had smoked >10 TC per day for >10 years and currently use a VP for >12 months. Smokers (n=19): smoking for >10 years and currently smoking >9 TC	Oxidative stress 8OHdG: NS diff. between groups. Inflammation IL-8: stat. sig. higher in vapers compared with smokers' group (p=0.001). Stat. sig. lower in vapers compared with non-users' group (p=0.001). TNF-α: stat. sig. higher in smokers compared with vapers' group (p=0.001).	8/20

Author, year of publication, country	Last follow-up (exposure length[1]) or study design	Participants' characteristics	Interventions/groupings	Study findings	Overall risk of bias[2]
			per day. Non-users (n=19): former smokers who had smoked >10 TC per day for >10 years and currently do not smoke for >12 months.		

Notes: [1] Exposure length: A—acute (1 to 7 days), S-M—short-to-medium (8 days to 12 months), L—long-term (over 12 months).

[2] Risk of bias measured using different tools for different study designs: RCTs & cross-over studies—RoB2 risk of bias tool; non-randomised longitudinal studies—ROBINS-I risk of bias tool; cross-sectional studies—BIOCROSS risk of bias tool.

8OhdG—8-hydroxy-2'-deoxyguanosine; A – acute exposure; CO – carbon monoxide; CRP – C-reactive protein; FMD – flow-mediated dilation; FU – follow-up; HDL—high-density lipoprotein; IL-6—interleukin-6; IL-8—interleukin-8; L – long exposure; LDL—oxidized low-density lipoprotein; LTA – light transmission aggregometry; MCP-1—monocyte chemoattractant protein 1; MDA—malondialdehyde; NS – non-significant; PGE-M –prostaglandin E2 metabolite; RCT – randomised controlled trial; ROS—reactive oxygen species; S-M—short-medium exposure; sICAM1—soluble intercellular adhesion molecule 1; Stat. sig. diff. – statistically significant difference; TC – tobacco cigarette; TNF-α—tumor necrosis factor α; VP – vaping product, nnVP – non-nicotine vaping product; WBC—white blood cell count.

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9 Cancers

9.1 Introduction

Tobacco smoking and cancers

Tobacco smoking is the largest preventable cause of several cancers and is associated with an increased risk of cancer recurrence, poor response to cancer treatment, and increased treatment-related toxicity (1). In England, tobacco smoking contributes to the highest proportion of preventable cancer cases (around 15% of all cases). The cancer types with the highest population attributable fractions for tobacco smoking are cancer of the lung (72%) and larynx (63%) (2). In England, tobacco smoking caused a quarter of all cancer deaths in 2020 (3). This is similar to other parts of the world (4, 5). We reported in chapter 4 that smoking prevalence among adults in England is currently around 12.7% to 14.9%. The smoking status among cancer survivors in the UK is poorly recorded (1) and differs according to type of cancer diagnosis. US cross sectional surveys suggest that around two-thirds of cancer survivors who regularly smoked prior to their cancer diagnosis continued to smoke (6) and the odds of continued smoking were twice as high among survivors of smoking-related cancers compared with survivors of non-smoking-related cancers (7). Stopping smoking at the time of a cancer diagnosis improves outcomes, including reduced risk of developing a second primary cancer and reduced treatment toxicity (8).

There is very little research about vaping prevalence among cancer survivors or those newly diagnosed with cancer, and we were unable to identify any studies conducted in the UK. A population wide survey in the US (9) reported current smoking prevalence of 12.7% among cancer survivors compared with 18.5% of non-cancer participants (though it is not clear if the non-cancer participants were otherwise healthy in this study); vaping product use was reported in 3.8% of cancer survivors compared with 5.7% of non-cancer participants. Young adult cancer survivors (aged 18 to 44) reported the highest rates of current cigarette smoking (27.9%) and current vaping product use (11.8%) (9). As with smoking, prevalence of vaping differs by type of cancer diagnosis (10).

Two studies conducted in the UK to date have surveyed health professionals who treat cancer patients regarding their attitudes and clinical practice about advising patients who smoke about vaping (11, 12). Participants reported that patients view clinicians as a source of guidance about vaping, yet few clinicians felt confident about advising patients (11). Knowledge about smoking cessation and vaping products, engagement in smoking cessation practices with patients that smoke, belief in effectiveness of vaping products and feeling comfortable discussing vaping with their patients were associated with recommending vaping products to people with cancer who smoke (12).

How tobacco smoking affects cancer risk

Most people who vape have a history of smoking. So, any estimate of risk to health among people who vape needs to take this into account. Each puff of a combusted tobacco cigarette contains a mixture of thousands of compounds, including around 70 well-established human carcinogens, such as some tobacco specific nitrosamines (TSNAs), volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs) and heavy metals. These carcinogens and other constituents in tobacco smoke can damage DNA and interrupt the natural repairing process of DNA damage. The accumulation of DNA damage in the same cells over time can lead to cancer (13). Long-term tobacco smoking results in repeated exposure to multiple carcinogens, the formation of DNA adducts and multiple mutations in critical cancer control genes (14). Also, components of tobacco smoke have potential co-carcinogenic or tumour-promoting activity and also contain a number of cytotoxic components, such as reactive aldehydes and carbonyls, which are capable of damaging lung cells and triggering inflammation (15). Carcinogen biomarkers — DNA adducts, protein adducts and metabolites can provide objective measures of carcinogen uptake and cellular changes in people who exposed to tobacco products. DNA adducts (a covalent binding product of a carcinogen or related substance or its metabolite to DNA) potentially provide the most direct link to cancer, but there are problems with measuring this, because of low concentrations and non-quantitative methods (16, 17). Urinary metabolites, which are easier to collect and measure can provide important information about carcinogen dose and metabolism (16). For comprehensive overviews of how combustible and smokeless tobacco use leads to cancers, Hecht (16) and Hecht and Hatsukami (14) provide excellent reading.

How vaping might affect cancer risk

The National Academies of Sciences, Engineering and Medicine (NASEM) report on the Public Health Consequences for E-Cigarettes (18) suggested several possible biological pathways for how vaping may theoretically influence the development of cancer. Exposure to toxicants in vaping products (for example, aldehydes) may cause inflammation, leading to cytotoxicity and cell death, potentially influencing tissue repair and mitogenic (a type of cell division) response. Toxicant exposure might also theoretically lead to reactive oxygen species and/or be converted to reactive intermediates that bind to DNA. This may cause damage to DNA and no or incorrect repair to DNA. NASEM (18) hypothesised this may then lead to activation of oncogenes (mutated genes that contribute to the development of a cancer) and/or loss of function of tumour suppression genes (normal genes that slow down cell division, repair DNA mistakes, or tell cells when to die). When tumour suppressor genes do not work properly, cells can grow out of control, which can lead to cancer.

Findings of a US Food and Drug Administration sponsored workshop on biomarkers of potential harm (BoPH) associated with tobacco and nicotine products (19) also suggested

biomarkers such as DNA adducts play a central role in carcinogenesis, and gene expression of the bronchial airway epithelium may serve as an early diagnostic biomarker for lung cancer in relation to smoking and may have relevance to the use of vaping products. Advances in the study of epigenetics have also contributed to our understanding of smoking and vaping. Epigenetics is the study of how behaviours and the environment can cause changes that affect the way our genes work (20). While genetic changes can alter which protein is made, epigenetic changes affect gene expression to turn genes 'on' and 'off'. Types of epigenetic changes include DNA methylation and non-coding RNA (methylation turns genes 'off' and demethylation turns genes 'on') (20). DNA methylation is a type of epigenetic modification involving the addition of methyl groups to the DNA which influences how the underlying sequence is interpreted and expressed. Although smoking leads to an overall decrease in DNA methylation, several critical genes such as p16 and p53 become hypermethylated in smokers, this could potentially eventually lead to uncontrolled cellular divisions and failure to properly regulate the cell cycle, leading to cancer (21). Epigenetic changes can be reversible and do not change your DNA sequence. DNA methylation is associated with smoking and Cg05575921 methylation in particular, appears to have a dose-dependent relationship and some, but not all altered DNA methylation is reversible after stopping smoking (19, 22).

A recent UK study by Richmond and others including 350 smokers, non-smokers and exclusive vapers suggests the DNA methylation profiles of vapers is less pronounced than that of smokers (23). DNA methylation (tested in salivary samples) at 13 cytosine-phosphate-guanine sites (CpGs) was associated with smoking at $p < 1 \times 10–5$ and one at $p < 5.91 \times 10–8$. Seven CpGs were associated with vaping at $p < 1 \times 10–5$ and none at $p < 5.91 \times 10–8$. There was strong enrichment of known smoking-related CpGs in the smokers but not the vapers. Richmond and others reported than vaping does not impact saliva methylation in the same way as cigarette smoking. Unlike for smoking, the methylation profile for vaping did not replicate in independent samples and was not able to discriminate cancer from normal tissue. (Please note, this study was published after our search end date and is therefore not included in the systematic review findings below.)

9.2 Summary of previous reports about the effect of vaping on cancer risk and outcomes

In the methods chapter (chapter 2) we explain the rationale for summarising these reports. The summary of reports and our systematic review below include human, cell, and animal studies. We give priority and most weight to human studies. We also include findings from cell and animal studies for completeness and, where indicated, note their limitations and lack of transferability to humans.

Previous evidence reviews on vaping, commissioned by Public Health England (PHE)

In our 2018 report (24) (which was not a systematic review but included literature until mid-August 2017) we included a study by Stephens (25) who adapted the method used in Fowles and Dybing (26) to model the relative harm caused by inhaling aerosols from various vaping products or a licensed nicotine inhalator compared with cigarette smoke. Published chemical analyses of these aerosol components were combined with estimates of their cancer potencies (unit risks) from the literature to compute model lifetime cancer risks based on daily consumption estimates. Unit risk predictions from vaping products were largely found to be a small fraction of those of smoking (<1%). Where findings exceeded 1% of the equivalent risks of smoking, this could be related to unrealistic use of vaping products for example, dry puffs and were therefore largely avoidable by the user. We concluded that the published evidence at the time of writing the report regarding the presence or absence of chemicals in aerosols from vaping products, suggested people who had switched from smoking to vaping were exposed to much lower levels of carcinogens and toxicants, many similar to those in nicotine replacement therapy (NRT) users (24).

The National Academies of Sciences, Engineering and Medicine report on the Public Health Consequences for E-Cigarettes

The NASEM report (18) (which searched the literature to the end August 2017) included 2 human studies relating to self-reported cancer in vapers, 2 about oxidative stress and inflammation and 2 case studies. NASEM reported studies were of poor quality, had small sample sizes and had not considered tobacco smoking history and potential confounding. A further 7 studies involving human cell lines or animals were included. The NASEM committee concluded there was no available evidence whether vaping was associated with intermediate cancer endpoints in humans, either when compared with tobacco smoke or no use of tobacco or vaping products. The Committee found substantial evidence that chemicals present in vaping product aerosols were capable of mutagenesis and DNA damage but concluded there was limited evidence that aerosols from vaping products were actually mutagenic or caused DNA damage in humans, human cell lines or animals (18). NASEM also stated ‘When the evidence is viewed in total, while there is a biological rationale for how nicotine could potentially act as a carcinogen in humans, there is no human evidence to support the hypothesis that nicotine is a human carcinogen. While it is biologically plausible that nicotine can act as a tumour promoter, the existing body of evidence indicates this is unlikely to translate into increased risk of human cancer’ (18).

The Committee on Toxicity Statement on the potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems

In collaboration with the Committee on Carcinogenicity (COC) and Committee on Mutagenicity (COM), 14 human cell and animal studies on the genotoxicity, oxidative stress, DNA damage and cytotoxicity (identified in the literature up to mid-2019) were considered by the Committee (27). The COC concluded that the relative risk of use of vaping products compared to tobacco cigarettes appeared to be lower, but there was still some risk associated with the chemicals and particles in the emissions from vaping products. The COM conclusions indicated a lack of consistency in the evidence base depending on the type of study. They suggested the quality of the studies did not provide an evidence base to allow the interpretation of preliminary associations between the use of vaping products and the risk of cancer in humans.

9.3 Findings from the systematic review

As outlined in chapter 2, our systematic review addressed 2 aims:

1. What effect does vaping and second-hand exposure to vaping products have on biomarkers that are associated with the risk of cancer?

2. What are the effects of vaping among people with existing cancer on disease outcomes?

We explain the methods for the systematic review in chapter 2. Quality assessments and the funding source of each study can be found in the appendices.

Summary of biomarkers of exposure with relevance to cancer

Chapter 7 includes tables of study characteristics, study findings, meta-analyses and narrative synthesis identified from the systematic review about several biomarkers of exposure that are associated with cancer risk in smokers, that is, some TSNAs, VOCs, PAHs and metals. Here we briefly summarise biomarkers of exposure that are classified as Group 1 carcinogens (carcinogenic to humans), by The International Agency for Research on Cancer (IARC) (28) in vapers compared with smokers or smokers who switched to vaping, as well as studies that compared those who were exposed to vaping compared with non-use. We refer the reader to chapter 7 for a more detailed overview of biomarkers of exposure.

Tobacco specific nitrosamines (TSNAs)

TSNAs are some of the most potent carcinogens in tobacco and the following are classified as Group 1 carcinogens: 4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone

(NNK) and its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), as well as N'-Nitrosonornicotine (NNN) (28).

Acute, short- and medium-term changes in urinary levels of NNAL were measured in 6 randomised control trials (RCTs) (29-34). We pooled data from 3 of these RCTs (30, 32, 34).The average urinary NNAL levels were statistically significantly lower among vapers than smokers' groups in a meta-analysis (log transformed mean difference (LMD): -0.87, 95% CI: -1.29, -0.45; 313 participants); the geometric mean NNAL levels were approximately 58% lower among vapers than among smokers (Geometric Mean Ratio (GMR): 0.42, 95% CI: 0.28, 0.64), though heterogeneity was high (I^2 = 88%). The other 3 RCTs reported lower NNAL levels in vapers compared with smokers, though some vapers had not completely stopped smoking (29, 31, 33).

In longitudinal studies, Goniewicz and others (35) reported a statistically significant reduction by approximately 78% in urinary NNAL levels at 2 weeks follow-up and Pulvers and others (36) reported approximately 96.6% reduction in NNAL levels 4 weeks after switching from smoking to vaping. Average levels of NNAL also dropped for the first few months after smokers switched to VP use in a study by Walele and others (37). However, NNAL levels did not significantly change in one acute exposure study with a follow up of 3 and 5 days (38) and in 2 cross-over studies (39, 40). Out of 11 cross-sectional studies that measured urinary levels of NNAL in vapers and smokers, levels were significantly lower by approximately 74% to 98% in vapers compared with smokers in 9 studies (41-48).

Twelve studies compared levels of NNAL between vapers and non-users (42-46, 48-54). Eight studies reported levels to be around 1.69 times (45) to 42 times (44) significantly higher among vapers compared to non-users.

Across cross-sectional studies that measured urinary NNAL, vapers' levels were approximately between 2% and 70% and non-users' levels were approximately between 0% and 37% of NNAL levels detected among smokers (chapter 7, figure 51).

Acute changes in urinary levels of NNN were measured in 3 RCTs (32-34), that also had measured NNAL. We pooled data from 2 of these studies (32, 34). The average urinary NNN levels were statistically significantly lower among vapers than smokers' groups in the 2 RCTs (LMD: -2.26, 95% CI: -2.55, -1.97; 225 participants); the geometric mean NNAL levels were approximately 90% lower among vapers than among smokers (GMR: 0.10, 95% CI: 0.08, 0.14) and heterogeneity was moderate (I^2 = 36%). An RCT by McEwan and others (33) also reported statistically significant reductions in urinary NNN levels 7 days after switching from smoking to vaping. Four cross-sectional studies also measured urinary levels of NNN in vapers compared with smokers (42, 48, 49, 53) one of which found significantly lower levels in vapers compared with smokers (42).

Four studies reported comparisons of NNN levels between vapers and non-users (42, 48, 49, 53). One study reported statistically significant differences between the groups, with

vapers having 81% higher levels in urinary samples compared to non-users (42). Another reported no difference between urinary levels, however reported salivary NNN levels to be on average 58 times higher among vapers compared to non-smokers; however, the reported variation was wide, sample size was small and differences were not tested for statistical significance (49). Two other studies reported higher levels in vapers than non-users, though neither study tested the comparisons for statistical significance (48, 53).

Across cross-sectional studies that measured urinary NNN, vapers' levels were approximately between 15% and 38% and non-users' levels were approximately between 0% and 16% of NNN levels detected among smokers (42-50) (chapter 7, figure 51).

Volatile organic compounds (VOC)

Benzene, 1,3-Butadiene and formaldehyde are VOCs that have been classified by IARC as human carcinogens (28). Regarding benzene, urinary levels of S-phenyl mercapturic acid (S-PMA), a metabolite of benzene, was reported in 11 studies; 2 RCTs (32, 33) and 2 longitudinal studies (35, 36) (that also measured the above TSNAs) reported average reductions of 87% to 90% over 4-weeks, within vapers' groups following switching from smoking. One cross-over study (55) reported 3.21 times higher S-PMA levels after acute exposure from smoking a cigarette than 15 puffs from a cartridge or pod vaping product or 10 puffs from a tank product. One longitudinal study (37) found a decrease within 4 weeks of smokers who switched to vaping. Four cross-sectional studies (42, 46, 54, 56) found significantly lower levels or urinary S-PMA in vapers compared with smokers. Another cross-sectional study also found lower urinary levels of (S)PMA in vapers than smokers, but this was not tested for statistical significance (57).

One longitudinal study (58) and 2 cross-sectional studies (46, 59) assessed the benzene metabolite trans,trans-Muconic acid (MU) and one study (57) assessed PHMA; all found significantly lower levels in vapers compared with smokers.

Six studies reported on levels of S-PMA among vapers and non-users (42, 46, 47, 54, 56, 57). Levels were reported to be no different among adolescent vapers and non-users (54). Among adult samples, levels were reported to be between approximately 14% lower (57) and 167% higher (56) among vapers compared to non-users, though the comparisons were not statistically significantly different. Urinary MU levels were reported to be on average 52% higher among vapers compared to non-users (59), and on average 58% lower among vapers compared to ex-smokers who used NRT (46). Both comparisons were not statistically significant.

Across cross-sectional studies that measured urinary benzene biomarker S-PMA, vapers' levels were approximately between 33% and 124% and non-users' levels were approximately between 13% and 123% of S-PMA levels detected among smokers (42, 46, 47, 56, 57). Vapers' levels of biomarker MU were approximately between 70% and 159%

and non-users' levels were between 105% and 168% of those reported among smokers (46, 59) (chapter 7, figure 28).

Monohydroxybutenyl mercapturic acid (MHBMA) and dihydroxybutylmercapturic acid (DHBMA) are 2 urinary biomarkers of exposure to 1,3-Butadiene that are carcinogenic in humans. Three RCTs reported on urinary MHBMA levels in smokers who switched to vaping or continued smoking (32-34). We pooled data from 2 of the RCTs (33, 34). The average MHBMA levels were statistically significantly lower among vapers than smokers in 2 RCTs (LMD: -1.80, 95% CI: -3.35, -0.24; 225 participants); the geometric mean MHBMA levels were approximately 83% lower among vapers than among smokers (GMR: 0.17; 95% CI: 0.04, 0.79) and heterogeneity was high (I^2 = 98%). A cross over study by St. Helen and others (55) reported 5.8 times higher MHBMA levels after smoking a cigarette than 15 puffs from a cartridge or pod vaping product or 10 puffs from a tank vaping product. We also pooled data from 2 longitudinal studies and found the average urinary MHBMA levels were statistically significantly lower among vapers than smokers (LMD: -4.46, 95% CI: -8.87, -0.05; 53 participants); the geometric mean MHBMA levels were approximately 99% lower among vapers than among smokers (GMR: 0.011; 95% CI: 0.00014, 0.95) and heterogeneity high (I^2 = 96%). Another longitudinal study found DHBMA among vapers did not differ from levels among smokers (58). Five cross-sectional studies measured urinary DHBMA levels among vapers and smokers and all found significantly lower levels in vapers (42, 46, 47, 56, 57, 59). A further cross-sectional study found lower DHBMA levels in vapers than smokers but did not conduct statistical tests between groups (57).

Six studies reported levels of DHBMA among vapers and non-users (42, 46, 47, 56, 57, 59). Five studies found no statistically significant differences between vapers and non-users, with levels ranging between on average 25% lower (46) among vapers when compared to ex-smokers who used NRT, to 7% higher (56) among vapers compared to non-users. Three studies were pooled to assess urinary DHBMA (47, 57, 59). Combining the 3 studies, the pooled geometric mean urinary DHBMA level was 6% lower among vapers compared to non-users and was not statistically significant.

Across cross-sectional studies that measured urinary DHBMA, vapers' levels were approximately between 56% and 111% and non-users' levels were approximately between 52% and 115% relative to urinary DHBMA levels detected among smokers. Across studies that reported urinary MHBMA, vapers' levels were approximately between 13% and 35% and non-users' levels were between 7% and 32% of levels detected among smokers (42, 46, 47, 56, 57, 59) (chapter 7, figure 34).

Two longitudinal studies assessed changes in urinary formate levels (a metabolite of formaldehyde). After single use of vaping product, Lorkiewicz and others (60) did not detect changes in urinary formate levels after single use of a vaping product or smoking. Dawkins and others (60) reported higher urinary formate levels after using a 6 milligrams

per millilitre (mg/mL) nicotine e-liquid with an adjustable-power vaping product for a week than using an 18mg/mL nicotine e-liquid with the same type of vaping device for a week. Dawkins and others (60) concluded that formaldehyde exposure might increase during compensatory puffing behaviour with lower nicotine strength e-liquids. No cross-sectional studies reported levels of formate.

Polyaromatic hydrocarbons (PAHs)

Chapter 7 also includes PAHs and their respective metabolites, benzo[a]pyrene (metabolite: 3-hydroxy-benzo[a]pyrene (3-OH-B[a]P) and pyrene (1-hydroxypyrene (1-HOP)). The latter is classified as not carcinogenic to humans (28), but is included here as it is always a component of mixtures of other polyaromatic hydrocarbons that are carcinogenic (61). An RCT by Round and others (32) reported a significant reduction in urinary 3-OH-B[a]P in smokers who had switched to menthol or non-menthol flavoured vaping product groups. Round and others (32) and McEwan and others (33) reported statistically significant reduction in urinary 1-HOP levels after switching from smoking to vaping by an average of over 60%. A cross-over study (40) reported a statistically significant reduction of over 30% in urinary 1-HOP levels when using vaping products. A non-randomised longitudinal study (35) found a statistically significant increase in urinary 1-HOP levels of 11.7% 2 weeks after switching from smoking to exclusive vaping. As with other studies, follow up of participants is only very short term. Four cross-sectional studies reported on urinary levels of 1-HOP (42, 43, 51, 62), 2 of which found significantly lower levels among vapers than smokers (42, 43).

Three studies reported differences in urinary 1-HOP between vapers and non-users (42, 43, 62), with one study finding statistically significant higher levels of urinary 1-HOP among vapers compared to non-users, by on average 26% (42).

Across cross-sectional studies that measured urinary 1-HOP, vapers' levels were approximately between 52% and 67% and non-users' levels were approximately between 33% and 44% of 1-HOP levels detected among smokers 42, 43, 62) (chapter 7, figure 52).

Metals

We did not identify any RCTs or longitudinal studies that measured levels of metals in vapers. Our search identified 10 cross-sectional studies that measured urinary and/or blood levels of cadmium among vapers and smokers (42, 43, 51, 53, 63-68). Findings were mixed, with levels being lower, similar or higher in vapers compared with smokers. Two cross-sectional studies, one measuring urinary levels of arsenic (42) and one measuring blood levels of arsenic (66) both found higher levels in vapers compared with smokers.

When vapers were compared to non-users, one study reported that urinary levels of arsenic were around 2% lower among vapers than among non-users, which was not

statistically different (42). Levels of urinary arsenic among vapers were approximately 90% and levels among non-users' were approximately 73% of arsenic levels detected among smokers (chapter 7, figure 55). Another study reported a 100% higher level of blood serum arsenic among vapers compared to non-users, however there was wide variation between participants and the difference was not statistically significant (66).

Four studies compared levels of urinary cadmium among vapers and non-users. One study reported levels to be statistically significantly higher among vapers, by around 29%, when compared to non-users (42). Three studies reported differences ranging from 17% lower to 100% higher among vapers compared to non-users, however none of these differences were statistically significant (43, 63, 65).

Another 4 studies also reported levels of cadmium in the blood among vapers and non-users (64, 66-68) with little consistency in findings. Statistically significantly higher levels among vapers, of approximately 42%, were reported by one study (64). Another reported 25% lower levels among vapers compared to non-users, however this was not statistically significant (66). Studies using substantially larger sample sizes reported levels to be between 11% lower and 30% higher among vapers compared to non-users, however these studies did not test for statistical significance (67, 68).

Across cross-sectional studies that measured urinary cadmium, levels among vapers were approximately between 48% and 104% and levels among non-users were between approximately 52% and 125% of cadmium levels detected among smokers (42, 43, 64, 65) (chapter 7, figure 55).

Summary

To summarise, findings from the RCT's and longitudinal studies broadly agree that exposure levels to the TSNAs NNAL and NNN are substantially reduced after smokers switch to vaping only. Most of the cross-sectional studies support this. As discussed in chapter 7), longer follow-ups are needed to account for the biological half-life of NNAL (approximately 10 days) (42) to 18 days (69) which indicates that this biomarker can be used to detect tobacco smoke exposure for 6 to 12 weeks after stopping smoking.

Findings from RCTs and longitudinal studies also broadly agree that exposure levels of the VOCs, 1-HOP, benzene and its metabolites S-PMA, MU and MHBMA are substantially reduced after smokers switch to vaping only.

Important absences from the data are some of the low molecular weight aldehydes, especially formaldehyde that can be generated during vaping especially at abnormally high temperatures. This was assessed in only 2 studies. The lack of validated biomarkers for these compounds is largely a consequence of their exceptional reactivity which tends to inhibit the formation of stable biomarkers amenable to conventional measurement techniques.

Biomarkers of the metals (which our review identified were only measured in cross sectional studies) do not strongly reflect differences in exposure to vaping and smoking. This is probably a function of the difficulty in running exposure experiments that involve compounds with very long half-lives. Also, as the design of vaping products have evolved over time, exposure to metal components will likely have changed depending on when the study was conducted, and the device type participants used.

Compared with non-use of tobacco or vaping products those who were exposed to vaping had higher levels of the above biomarkers, though as discussed in chapter 7 studies assessing relative and absolute exposures did not take into account other potential exposures with relevance to cancer, such as nitrosamines and volatile organic compounds, and the studies did not always control for the effects of past tobacco smoking. The reader is also referred to chapter 7 for further information about other studies of exposure biomarkers classified by IARC as group 2 and 3 carcinogens.

Biomarkers of potential harm cutting across several diseases

Our systematic review also identified several studies that measured inflammation and oxidative stress related to vaping products (chapter 8). Here we focus specifically on the biomarkers of inflammation and oxidative stress identified by Chang (17) that have relevance to cancer, though inflammation and oxidative stress play a role in the development and outcome of several smoking related diseases such as cardiovascular and respiratory diseases and hence are in a separate chapter of biomarkers that cut across several diseases. Tables of study characteristics and findings can be found in chapter 8, tables of the risk of bias assessments and study funders can be found in the appendices.

Studies in humans: inflammation and oxidative stress

Study characteristics

The search identified 21 studies on inflammatory and/or oxidative stress biomarkers that have relevance for cancer risk (notably C-reactive protein; Interleukin-6 Interleukin-8; Prostaglandin E2 metabolite and oxidized low-density lipoprotein). Two were RCTs (70, 71), 4 were longitudinal intervention studies (37, 72-74) and 15 were cross-sectional (43, 75-88); 3 of which were dental studies (87-89).

C-Reactive Protein (CRP)

Seventeen studies included inflammatory biomarkers with relevance to cancer risk. Of these, 7 assessed CRP. In studies that compared vaping with smoking, George and others (70) reported there was a non-statistically significant difference in highly sensitive-(hs)-CRP plasma levels between groups randomised to 4 weeks of ad lib use of a 16mg/mL vaping product compared to a 0mg/mL vaping product or the control group who continued to smoke. In the cross-sectional studies, Perez and others (43) reported that plasma hs-

CPR levels were lower in vapers compared with smokers. Moon and others (75) reported plasma levels were higher in vapers compared with smokers. Faridoun and others (78) found higher salivary CPR levels in smokers than vapers. However, all the differences were statistically non-significant.

In studies that compared vaping to no smoking or no vaping (non-use), Perez and others (43) reported that serum highly sensitive CPR levels were lower in vapers compared with non-users. Moon and others (75) reported plasma levels were higher in vapers compared non-users. Faridoun and others (78) found higher salivary CPR levels in vapers than non-users; Singh and others (80) and Boas and others (86) reported similar plasma CRP levels between vapers and non-users. Again, all the differences were statistically non-significant. In an acute exposure interventional study, Chatterjee and others (73) found a statistically significant increase in CPR serum levels an hour after 3 minutes of vaping a non-nicotine vaping product, in 31 non-smokers. Previous smoking and vaping histories or verification of their current status were inconsistently defined across studies or missing.

Interleukins (IL-6 and IL-8)

Ten studies assessed Interleukin-6 (IL-6), with mixed findings. In studies that compared vaping with smoking, Benowitz and others (74) found those who were exposed to ad lib use of their own brand of vaping product or continued to smoke had no difference in IL-6 levels. Two cross sectional studies reported significantly higher levels in smokers compared with vapers (76, 77), whereas 2 other cross sectional studies found no significant differences between groups of vapers and smokers (43, 78).

There was a non-significant difference in a study by Song and others (71) between a group of people who had not smoked more than 100 cigarettes in their lifetime or vaped in the past year and were randomised to either vape a 50% propylene glycol/vegetable glycerine (PG/VG) ratio (non-nicotine, and non-flavoured) e-liquid for 4 weeks or a control non-exposure group. In a cross-over study by Benowitz and others (74) IL-6 levels were significantly lower in a non-use group compared with those who were exposed to ad lib use of their own brand of vaping product or continued to smoke and no difference between the smoking and vaping group. In cross sectional studies, Singh and others (80) found IL-6 levels were higher in a vaping group compared with a non-using group. AlQahtani and others (87) found IL-6 levels were higher in concurrent smokers and vapers than a non-using group. Kotoulas and others (72) assessed smokers with and without asthma, exposed to 5 minutes of vaping a nicotine containing vaping product, and did not find a significant difference within groups nor between those with and without asthma.

Eight studies assessed Interleukin-8 (IL-8) and also found mixed results. In studies that compared vaping with smoking, Benowitz and others (74) reported no difference between vaping and smoking groups. In cross sectional studies, Song and others (77, 78) found no difference between vaping and smoking groups. In study participants with periodontal disease (88) mean levels of IL-8 were significantly lower in the smoking group compared

with a vaping group and a former smoking group, and levels in vapers were lower than former smokers.

In studies that compared vaping with no use, Song and others (71) found a non-significant difference between groups randomised to either vape PG/VG e-liquid for 4 weeks or a control non-exposure group. Benowitz and others (74) reported statistically significantly higher levels in a vaping group than in a non-use group. Singh and others (80) found statistically significant higher levels in a vaping group compared with non-users. Song and others and Ashford and others found no difference between the vaping and non-using groups.

In smokers with and without asthma, exposed to 5 minutes of vaping a nicotine containing vaping product, Kotoulas and others (72) did not find a significant difference within groups nor between those with and without asthma.

Prostaglandin E2 metabolite (PGE-M)

PGE-M is associated with several cancers has been suggested by Chang and others (19) to be a promising inflammatory biomarker. One study found statistically significant lower levels of this marker in vapers compared with smokers and no difference between vapers and non-users and concurrent smokers and vapers (81), Singh and others (80) found no difference between vapers and non-users.

Oxidized low-density lipoprotein (LDL)

LDL has been found to play a role in smoking and lung cancer as well as cardiovascular disease. Eight studies assessed this marker, 3 of which also assessed inflammatory markers (70, 75, 86). The majority of studies found no significant difference in levels between vapers and smokers (70, 75, 84, 86, 90), and between vapers and non-users (82, 85). Walele and others (37) reported within group significant differences in smokers who were exposed to ad lib use of a 16mg/mL vaping product at 6-, 12- and 18-months follow-up.

Summary of findings

Available studies of the CRP inflammatory biomarker do not demonstrate any systematic relationship with mixed evidence of differences (or no difference) in levels between vapers and smokers and non-users. Some studies found IL-6 levels to be higher in smokers and vapers compared with non-users and that the levels in vapers were either lower than in smokers or there was no significant difference. For IL-8, in a few studies in which significant differences have been observed both vapers and smokers have higher levels compared with non-users, with some evidence that IL-8 levels in vapers may be higher than in smokers. On the basis of 2 studies, PGE-M, a promising inflammatory biomarker for smoking-related diseases, is present at significantly elevated levels in smokers

compared with vapers in whom it is present at similar levels to non-users. LDL does not appear to be sensitive to smoking/vaping status and non-use.

With the exception of CRP and LDL the biomarkers investigated in this review show some evidence that an inflammatory response is induced by vaping and smoking, and that the response is less in people exposed to vaping than smoking. There is clearly a need to develop more robust indicators of potential harm to better assess the risk of cancer due to vaping compared with smoking, in particular there is a need for well-designed experiments to establish whether PGE-M is a useful biomarker of potential harm for cancer in different forms of nicotine delivery.

Biomarkers of potential harm with specific relevance to cancer risk

Studies in humans: gene expression, non-coding RNAs and DNA methylation

Study characteristics

Our search identified a further 8 studies in humans that provide information on cancer risk (Tables 1a and 1b). Study designs included 2 RCTs (71, 91), one longitudinal intervention study (92), and 5 cross sectional studies (93-97), all conducted in the US. Sample sizes ranged from 3 (92) to 435 (94). Ages ranged from 18 to 65 years, though across studies, most of the participants who vaped were in their 20s. Funding sources for these studies can be found in table 5 in the appendices.

Risk of bias in included studies

Risk of bias tables can be found in tables1 to 4 in the appendices. The RCT by Song and others (71) was rated as low risk of bias for deviations from intended interventions, missing data and outcome measurement for of outcomes, and rated as ‘some concerns’ for the randomisation process and selection of reported results. Staudt and others (91) was rated as low risk of bias for missing outcome data and outcome measurement and some concerns for randomisation process, deviations from the intervention and selection of reported results. The longitudinal study and cross-sectional studies were rated as moderate risk of bias.

Study findings

The studies are narratively synthesised. Two RCTs and one longitudinal study assessed changes in gene expression (71, 91, 92) (table 1a). They did not include a comparison group of current smokers, so are unable to tell us anything about vaping in relation to smoking for biomarkers of potential harm related to cancer and provide mixed evidence of changes in gene expression of people exposed to vaping.

An RCT by Staudt and others (91) included 10 people with no self-reported history of smoking or vaping product use (verification of no recent use was confirmed by urinary

nicotine and cotinine levels). The average age of participants was 40 years and half were female. Seven participants were randomized to using a vaping product with nicotine and 3 to the same type of vaping product without nicotine (characteristics of propylene glycol (PG) to vegetable glycerine (VG) ratio, flavouring or nicotine strength of the e-liquid were not reported). Participants were instructed to inhale 10 puffs, wait 30 minutes, then inhale another 10 puffs.

Genome-wide gene expression profiles were assessed by mRNA-sequencing from small airway epithelium brushings; 71 genes were significantly altered following acute exposure to a vaping product with nicotine including 19 that were upregulated and 52 downregulated. Pathways significantly affected included several downstream targets of p53, including up-regulated genes (Endothelin 1; Angiomotin-like-2; large tumour suppressor kinase and Rho family GTPase 3) and down-regulated genes (ATPase family, AAA domain containing 2; Guanine deaminase; Marker of proliferation Ki-67; NDC80 kinetochore complex component and Ribonucleotide reductase M2). The p53 signaling pathway regulates several cellular functions including apoptosis, cell cycle arrest, and the DNA damage response and its activation is important in preventing development of tobacco smoke-induced lung cancer (91).

Sixty-five genes were significantly altered following acute exposure to a vaping product without nicotine, including 40 that were upregulated and 25 downregulated. Possible pathways implicated for the non-nicotine exposure group was not well defined by the authors, and they suggested that nicotine receptor pathway - Potassium channel, subfamily K, member 15 and Guanine nucleotide binding protein (G protein), beta polypeptide 1-like were implicated.

In the same study (91) genome-wide gene expression profiles were assessed by mRNA-sequencing of alveolar macrophages collected by bronchoalveolar lavage: 27 genes were significantly altered following acute exposure to nicotine, including 6 that were upregulated and 21 downregulated. Sixty-one genes were significantly altered following exposure without nicotine, including 25 that were upregulated and 36 downregulated. Staudt and others (91) reported that although no dominant pathways in the alveolar macrophages transcriptome data were evident, several individual genes known to be involved in macrophage physiology and host defence were affected by vaping exposure without nicotine including forkhead box M1 coronin-1A and prostaglandin E receptor 3, suggesting an altered immune response.

In another RCT, Song and others (71) assessed gene expression (microRNA, and mRNA from lung epithelial cells) among 30 people aged between 21 and 30 years who had never smoked or smoked fewer than 100 cigarettes in their lifetime and had not vaped within the past year. Fifteen participants were randomised to use a tank style vaping product that contained 50:50% PG/VG and was nicotine- and flavour-free. Participants were instructed to use the device at least twice per day, 20 puffs over 60 minutes each time, for a period of

4-weeks. The other 15 participants were randomised to receive no intervention. Compliance to the vaping intervention was assessed by daily LED readouts of puff number transmitted via cell phone and the measurement for increases in urinary PG (which was significantly increased in the vaping group (P=0.0015), but not the control group (P=0.72). After 4-weeks of vaping there were no statistically significant changes in gene expression from lung epithelial cell brushings for either group and no differences between the intervention and controls groups, leading the authors to conclude that after one month of use, large magnitude changes in gene expression are likely not to occur from inhaling PG and VG.

Hamad and others (92) assessed gene expression of 84 genes related to DNA damage in blood and buccal (inner cheeks) samples of 3 participants (one female and 2 males) aged 18 to 59. Participants were daily vapers who had not smoked for the previous 2 months; (eligibility criteria included at least 100 cigarettes in their lifetime and they must have quit at least 2 months before participation in the study). How long participants had been vaping or if they had been former smokers and for how long is not reported. Participants used their own tank style vaping product and were exposed to 50:50 PG/VG and 3 to 6mg/mL nicotine in 3 separate visits to the lab (they were presumably vaping between study visits, but this isn't clear). During each lab visit when samples were collected, subjects were asked to vape 20 puffs (3 seconds puff every 60 seconds, for a total of 20 puffs over 20 minutes). There was no comparator group. Five genes were significantly upregulated (flap structure-specific endonuclease 1; apoptosis inducing factor mitochondria associated 1; X-ray repair cross complementing 2; three prime repair exonuclease 1 and tumour suppressor TP53 gene. In blood, there was a significant downregulation of N-methylpurine DNA glycosylase (a repair gene). In both buccal and blood samples, the DNA replication, recombination, repair pathway was the major pathway activated by exposure to vaping. Changes were associated with puff volume and flow rate. The greater expression of several genes was associated with the greater puff volume and flow rate, particularly TP53.

Five cross-sectional studies assessed gene expression or DNA methylation among people who vaped, smoked and who were non-users (table 1b). Caliri and others (93) assessed 45 people divided equally into 3 groups of exclusive vapers, smokers, and non-users, matched for age (mean ~29 years) and sex (13% female). Participants in the vaping group were included if they had not smoked in the past 6 months and the smoking group had to have smoked for a minimum of one year and not vaped for 6 months. It is not clear how many in each group actually had previous smoking and vaping histories. Compared with the non-users, vapers and smokers showed significant loss of methylation in LINE-1 repeat elements and significant reductions in 5-hmC levels, but there was no difference between smokers and vapers. There was no statistically significant difference in changes in transcription of DNA methyltransferases among all 3 groups.

In a larger cross-sectional study of 112 people who smoked, 35 who vaped, 19 who used smokeless tobacco, and 269 non-users, Andersen and others (94) reported that cigarette smoking was associated with a dose dependent demethylation of cg05575921, but vaping or smokeless tobacco use did not demethylate cg05575921. Groups were more carefully selected than in the other cross-sectional studies, and in all groups self-reported nicotine and tobacco use was verified by urinary levels of cotinine, 2-cyanoethylmercapturic acid and expired breath carbon monoxide, anabasine and anatabine. Vaping status across groups was measured by PG levels.

Two cross-sectional studies (95, 96) drew their participants from multiple larger data sets (80, 98, 99) and compared the expression of long non-coding RNAs (lncRNAs) and microRNAs among those who exclusively vaped, exclusively smoked, waterpipe smokers, concurrent cigarette and waterpipe smokers and non-users. Participants and study groups in the Kaur and others (95) study were more clearly defined and explained than in the Singh and others (96) paper. lncRNAs (95) and microRNAs were differentially expressed between different types of nicotine groups and non-users whereas some microRNAs were common in both tobacco and vaping product users (96). Specifically, 9 microRNAs were differentially expressed in the vaping compared with the smoking group of which 5 were upregulated and 4 down regulated; whereas 17 microRNAs were differentially expressed in vapers in comparison with non-smokers (table 1b) (96). The authors reported the biological pathways involved for the vaping versus non-smoking group (as well as the non-smoking versus the smoking, waterpipe users and dual users of cigarettes and waterpipe) were beta1 integrin cell surface interactions, integrin family cell surface interactions, TRAIL signaling pathways. Endothelin biological pathways were implicated in the non-smoking versus vaping and dual users of cigarette and waterpipe smoking groups.

A cross sectional study by Corbett and colleagues (97) included 15 people who vaped, 9 who were smokers and 21 people who were former smokers. Mean ages were 35.7, 42.2 and 43 years for the respective vaping, smoking and former smoking groups and 37% were female. People in the vaping group were defined as former smokers who had been tobacco abstinent for a minimum of 3 months and had used any type of vaping product at least 6 days per week for at least one month. People in the smoking group were defined as current smokers with a minimum of 5 cigarettes per day and had used vaping products no more than twice in their life. Former smokers were defined as those who had been abstinent from tobacco for a minimum of 3 months and not used any form of nicotine replacement therapy. Status was verified by carbon monoxide and cotinine levels. Gene-expression profiling of bronchial epithelial cells collected during bronchoscopy was conducted and 3,165 genes were found to be statistically significantly differentially expressed. The gene expression among the vaping group was more similar to the former smoking group and statistically significantly different to the smoking group. Differential expression of genes in relevant gene-expression pathways, specifically glutathione and xenobiotic metabolism, tumour necrosis factor receptor 2 signaling, were distinct in smokers but not significantly different between those who vaped and former smokers.

Seventy-nine genes were up- or down-regulated concordantly among the cells of the participants who vaped and currently smoked. Pathway enrichment included interleukin receptor complexes (upregulated) and axon guidance (downregulated). There were some unique effects related to expression of genes in the vaping group: 468 genes were significantly altered in the cells of people who vaped relative to those who were former smokers, significantly enriched for the Ribosome biogenesis which the authors suggested might reflect increased oxidant stress. Also unique to the vaping group was significantly enriched targets of ATF2, which may regulate inflammation in the lung. The authors concluded that vaping product use does not lead to alterations in the expression of the majority of genes that are altered by tobacco smoke, but that there is a group of genes whose expression is specifically altered in those who vape.

A cross-sectional study by Song and colleagues (77) included 15 people who vaped, 16 who smoked and 42 who had never smoked more than 100 cigarettes in their life or vaped in the previous year. The overall mean age of participants was 26 years (range 21 to 30) and 47% were female. Those in the vaping group had vaped for an average of 2.7 years (range 0.5 to 4), and 13 of the 15 had previously smoked for an average of 7.5 years (range 1 to 15). Most used tank type devices with an average of 10mg/mL of nicotine strength (range 1.5 to 36). Average cigarettes per day in the smoking group was 16 (range 10 to 20) and they had smoked for an average of 6.6 years (range 0.6 to 13). Total RNA was extracted from bronchial epithelial cells via bronchoscopy and assayed for gene expression. There were 2,452 differentially expressed transcripts corresponding to 2,093 unique genes across the 3 groups. The expression profiles of non-users were closely clustered and separated from smokers, while those from the vaping group and non-user group were more similar to each other. Vaping product users' gene expression was intermediate between the smoker and non-user groups for 93% of the 2,452 differentially expressed transcripts. There were 181 transcripts that were related specifically to vaping product use (higher or lower than both smokers and non-users); the top 10 transcripts were MUC5B (4 transcripts), MIC5AC, ZNF445, REEP1, ABHK4, LINC00589, and TMPRSS3. The most common canonical pathways for differentially expressed transcripts included smoking and/or lung cancer-related pathways such as xenobiotic metabolism signaling, NRF2-mediated oxidative stress response, aryl hydrocarbon receptor signaling, PXR/RXR activation, and LPS/IL-1 mediated inhibition of RXR function. Eleven differentially expressed transcripts genes that were found to be regulated by beta-naphtoflavone were hypomethalated, with the highest expression in smokers, lowest in non-users, and vapers in the middle. The most represented disease was cancer, encompassing 51 genes (91%, 51/56), which included 27 (53%, 27/51) involved in respiratory tumours.

In a subsample of 12 vapers, 10 smokers and 10 non-users, 451 differentially methylated CpGs corresponding to 273 unique genes were identified. Of the 451 differentially methylated CpGs, for 97%, the vaping group were intermediate between smokers and non-users. There were 14 CpGs relating specifically relating to the vaping group (higher or

lower than smokers and non-users) (lower levels: RHBDL2, TTC16, ZNF815, and 3 intergenic CpGs; higher levels for AMZ1, KRT12, NOX5/MIR548H4 co-localized, NRF1, and 4 intergenic CpGs). The most common canonical pathways for differential DNA methylation were xenobiotic metabolism signaling and colorectal cancer metastasis signaling. Song and colleagues concluded that the effect of smoking on the lungs may be partially reversible in smokers who switch exclusively to vaping. Song and others concluded that the results of the vaping group were found to be intermediate between smoking and non-use groups for biomarkers of inflammation, gene methylation and expression, including known smoking-related pathways. Biomarker levels among vapers were more closely related to those who had never smoked.

The lack of comparisons with current smokers in the RCTs and longitudinal study limits what we can infer about relative cancer risk. The cross-sectional studies, which all included people who smoked as comparison groups, reported either similar or more favourable effects of vaping than smoking on gene expression and DNA methylation. Compared with non-using groups, vaping was less favourable and appears to have some unique effects, separate to smoking. However, all the studies are limited by possibility that other important confounders may account for the results, such as the residual effects of smoking and additional exposures that may influence cancer risk, such as diet and environmental exposures and in the case of the cross-sectional studies, causality cannot be confirmed.

Table 1a. Human studies: biomarkers of potential harm with relevance to cancer risk - RCTs and longitudinal studies

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Interventions/groupings	Study findings	Risk of bias
RCT					
Song et al., 2020, US (71)	4 weeks (S-M)	n = 30 Non-smokers: self-reported healthy non-smokers, had smoked <100 TC in their lifetime and had not used a VP in the past year. Randomised to: 1) VP non-nicotine (n=15, median age 25 years, 53% female, 73% white) 2) Non-users (n=15, median age 27 years, 67% female, 73% white)	Vaping: (n=15): instructed to take 20 puffs over 60 minutes ≥2 times a day using a tank VP (Innokin iTaste) with 50/50% PG/VG e-liquid without flavour or nicotine. Non-use (n=15): no use of VP. Compliance to the VP intervention was assessed by daily LED readouts of puff number transmitted via cell phone and the measurement for increases in urinary PG. This was significantly increased in the intervention (P=0.0015) and not the control group (P=0.72).	Gene expression (mRNAs & miRNAs) collected by lung epithelial cell brushings after 4 weeks. mRNAs: NS difference between VP-non-nicotine and non-user groups. miRNAs: NS difference between VP-non-nicotine and non-user groups.	Some concerns
Staudt et al., 2018, US (91)	Single use (A)	n = 10 Self-reported never smokers validated by	Vaping nicotine (n=7): instructed to take 10 puffs on a cartridge VP (blu) followed by other 10 puffs	Gene expression. Small airway epithelium: VP with nicotine: Significantly altered= 71 genes (19 up-	Some concerns

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Interventions/groupings	Study findings	Risk of bias
		<2 ng/mL urinary nicotine and <5 ng/mL urinary cotinine. Good overall health, with no respiratory disease. Mean (SD) age: 40.2 (9.7), 50% females, 70% Black, 30% Hispanic. Randomised to (1) VP nicotine (n=7) (2) VP Non-nicotine (n=3)	after 30 minutes. Vaping, no nicotine (n=3): same regime with the same VP without nicotine. Assessments at baseline and 2 hours post 2nd exposure	regulated and 52 down-regulated) VP without nicotine: Significantly altered= 65 genes (40 up-regulated and 25 down-regulated). Alveolar macrophages: VP with nicotine: Significantly altered= 27 genes (6 up-regulated and 21 down-regulated). VP without nicotine: Significantly altered=61 genes (25 up-regulated and 36 downregulated.	
Longitudinal					
Hamad et al., 2021, US (92)	Longitudinal Single use (A)	n= 3 Vapers: Self-reported daily users of cartridge or tank VP with ≤6mg/mL nicotine eliquid. Had been using a VP ≥8 times a day and not smoked in the past 2 months.	Buccal and/or blood samples collected before and after exposure to VP on 3 separate visits. Vaping (n=3): 20 3second puffs with 60 second inter-puff interval for 20 minutes of own tank VP with 3mg/mL (n=1) or 6mg/mL (n=2) nicotine strength e-liquid during 3 separate	DNA damage and repair & gene expression in 84 genes assessed. Buccal samples: Five out of 84 genes were significantly upregulated (FEN1, AIFM1, XRCC2, TREX1, TP53). Blood samples: MPG was significantly downregulated. Greater gene expression was associated with greater puff volume and flow rate for several	Critical risk of bias

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Interventions/groupings	Study findings	Risk of bias
		Age range: 18-59 years, 1 female, 2 males.	visits. FU: 5-15 days	genes.	

Table 1b. Human studies: biomarkers of potential harm with relevance to cancer risk – cross-sectional studies

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Study findings	Risk of bias
Cross sectional				
Andersen et al., 2021, US (94)	NA	n = 435 Vapers (n=35): VP use ≥once a week for the past year, smoking <100 TC in lifetime, no cannabis and other tobacco use for at least 1 year. Mean age: 23.5, 59.8% females. Smokers (n=112): ≥5 pack-years, current smoking of ≥2 TC per day, no VP use. Mean age: 41.2, 62.8% females. Non-users: n=269) smoking ≤100 TC or	DNA methylation of cg05575921 assessed in blood. Compared with smoking (which was associated with a dose dependent demethylation of cg05575921, increased urinary CEMA and anabasine levels), VP or smokeless tobacco did not demethylate cg05575921. Mean (SD) cg05575921: VP: 84.78 (5.12) Smokers: 54.53 (2.83) Smokeless tobacco: 84.29 (4.60) Non-users: 86.78 (2.83) Vaping frequency over each time period was unrelated to cg05575921 methylation (all p > 0.05).	10

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Study findings	Risk of bias
		cannabis joints in lifetime, no products in the past year, Mean age: 32, 69.7% females. Smokeless tobacco (n=19): daily use, ≤100 TC in lifetime, no cannabis or tobacco in past year Mean age 36.6, 5% females.		
Caliri et al., 2020, US (93)	NA	n = 45 Vapers (n=15): self-reported current vaping for ≥3 times a week for ≥6 months, no smoking or other tobacco use in the past 6 months. Mean (SD) age: 29.3 (1.8), 13.3% females. Smokers (n=15): self-reported current smoking ≥3 times per week for >1 year, no use of other tobacco, or VP in the past 6 months. Mean (SD) age: 29.5 (1.8), 13.3% females.	Global DNA methylation and hydroxymethylation assessed by collecting 30 mL peripheral blood DNA methylation Stat. sig. losses in both VP (p=0.008) and smoker (p= 0.031) groups in comparison to non-users. Methylation levels of LINE-1 elements decreased in vaper (~18%) and smoker (13%) groups compared with non-users. NS diff. between VP and smoker groups in the methylation levels of LINE-1 repeats (p=0.802). DNA hydroxymethylation (5-hmC) VP (p= 0.049) and smoker (p= 0.003) groups had significant reductions in 5-hmC levels compared with non-users. The levels of 5-hmC in VP and smoker groups were reduced by ~66% and 81%, compared with non-users.	6

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Study findings	Risk of bias
		Non-users (n=15): self-reported ≤100 TC or ≤5 vaping sessions in lifetime, no use of TC or VP in the past 6 months. Mean (SD) age: 28.9 (2.1), 13.3% females.		
Corbett et al. (2019), US	NA	n = 45 Vapers (n=15) Mean (SD) age: 35.7 (10.4), 26.6% females. All former smokers – quit 8.7 months ago Smokers(n=9). Daily use of at least 5 CPD Mean (SD) age: 43 (10.7), 47.6% females. Former smokers (n=21). Time since quit: mean (SD) 67 months (117). Mean (SD) age: 42.2 (11.3), 50% females.	Bronchial airway epithelial cells collected by bronchoscopy: Differentially expressed genes =3165. Specific to VP use =468 genes VP and TC upregulated: (interleukin recptor complexes) CMKLR1, ESYT3, FCAR, LILRB3, MIP, NPBWR2, VSIG2. VP and TC downregulated: (Axon guidance) NCK2, SEMA5A, SLIT2. VP upregulated: (ATF2 targets) AAMP, ATP5G2, BANF1, CKS1B, CLPB, CTDP1, DAPK3, DDX49, EMC10, FAM83E, HDDC3, HIST1H4I, ID1, ING4, IRF2BPL, JMJD4, MARS, MPND, MRPL17, MRPL4, MUTYH, MZT2A, NOC4L, RBM15B, RPL10, RPL22L1, RPL39L, RPLP0, RPS14, RPS19BP1, RPS9, RRP1, SH3BPS5L, SNORA16A, SNORA21, SNORA24, SNORA57, SNORA9, SNORD104, SNORD15A, SNORD22, SNORD27, SNORD50A, SNORD60, SNORD76, SNORD81, SNORD95, STX4, VTRNA1-3, ZYX. VP downregulated: (RFX3 targets) C10ORF194, CD200, DYNLRB2, FAM81B, FBXL2, IQCG, KIFAP3,	16

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Study findings	Risk of bias
			MAP1B, MAPK10, PPP1R42, RCN2, RSPH1, SPEF2, ZCCHC11	
Kaur et al, 2020, US (95)	NA	n = 24 Vapers (n=6): self-reported daily use for ≥6 months and not using tobacco products. Smokers (n=6): self-reported smoking ≥10 TC per day for ≥6 months, without chronic illness. Waterpipe smokers (n=6): 1–2 waterpipe sessions per day for ≥past 6 months. Dual cigarette & waterpipe smokers (n=6): smoking and waterpipe smoking. Non-users (n=6): self-reported non-use of any tobacco products. Age range: 18–65 years, 50% females.	Long non-coding RNAs (lncRNAs). VP group vs smokers' group: 10 lncRNAs were downregulated and 12 showed increased expression (differences between groups are not reported). VP group vs non-users' group: 4-fold increase in the expression of BNIP3L, Bcl2 interacting protein 3-like protein in VP group compared to the non-smoking controls.	6
Singh et al., 2020, US (96)	NA	n = 48 Self-reported users of VP, waterpipe and TC	Gene expression (microRNAs) assessed in blood. microRNAs VP vs smokers: Significantly differentially altered=9 (5	8

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Study findings	Risk of bias
		and non-users. Participant information provided in different papers. Vapers (n=22); Mean (SD) age: 35.5 (12.2), 54.5% females. Smokers (n=26); Mean (SD) age: 46.7 (10), 50% females. Waterpipe smokers (n=12); Mean (SD) age: 32.8 (14.0), 30.8% females. Dual TC and waterpipe smokers (n=10); Mean (SD) age: 35.5 (12.2), 54.5% females. Non-users (n=26); Mean (SD) age: 33.9 (14.0), 57.7% females.	upregulated; hsa-miR-362-5p; hsa-miR-2355-5p; hsa-miR-532-5p; hsa-miR-144-5p; hsa-miR-30e-5p: and 4 downregulated; hsa-miR-365a-3p; hsa-miR-1299; hsa-miR-193b-3p; hsa-miR-186-5p). VP vs non-smokers: Significantly differentially altered=17 microRNAs (13 upregulated; hsa-miR-365a-3; hsa-miR-365b-3p; hsa-let-7f-5p; hsa-miR-1299; hsa-miR-21-5p; hsa-let-7i-5p; has-let-7a-5p; hsa-miR-30a-5p; hsa-miR-193b-3p; hsa-miR-100-5p; hsa-miR-423-3p; hsa-miR-30c-5p; hsa-miR-143-3p and hsa-miR-224-5p,4: 4 were downregulated;hsa-miR-362-5p; hsa-miR-29b-3p; hsa-miR-451a and hsa-miR-30e-5p).	
Song et al., 2020, US	NA	n = 73 Mean (range) age: 26 (21-30), 47% females. Vapers (n=15): self-reported. Mean age (range) 27 (21-30).	Gene expression Total RNA extracted from the bronchial brushings. Differentially expressed transcripts (DETs) =2,452. Unique genes differentially expressed= 2,093. 181 (7%) of transcripts were related specifically to vapers (higher or lower than both smokers and never-smokers); the top 10 transcripts were MUC5B (4 transcripts), MIC5AC, ZNF445, REEP1, ABHK4,	12

Author, year of publication, country	Last follow up (exposure length)	Participant's characteristics	Study findings	Risk of bias
		33% females. Smokers (n=16): self-reported. Mean age (range) 26 (21-30). 4% females. Non-users (never smokers) (n=42): smoked <100 TC in their lifetime and not used a VP for the past year. Mean age (range) 25 (21-30) 25% females.	LINC00589, and TMPRSS3. Gene expression in vaping groups were intermediate between smokers and non-users (never-smoking) groups for 93% of the 2,452 DETs. Genome-wide methylation from bronchial brushings (subset of 12 vapers, 10 smokers and 10 non-users) Differentially methylated CpGs=451, corresponding to 273 unique genes. Of the 451 differentially methylated CpGs, for 97%, the e-cig users were intermediate between smokers and never-smokers. 14 CpGs relating specifically relating to e-cig use (higher or lower than smokers and never-smokers) (lower levels: RHBDL2, TTC16, ZNF815and 3 intergenic CpGs; higher levels for AMZ1, KRT12, NOX5/MIR548H4 co-localized, NRF1, and 4 intergenic CpGs).	

Notes: AIFM1-apoptosis inducing factor mitochondria associated 1; CO – carbon monoxide; CRP – C-reactive protein; DBP – diastolic blood pressure; DU – dual users of tobacco cigarettes and vaping products; FMD – flow-mediated dilation; FEN1-flap structure-specific endonuclease 1; FU – follow-up; HR – heart rate; LTA – light transmission aggregometry; MDA – malondialdehyde; MPG -N-methylpurine DNA glycosylase; NS – non-significant; N-methylpurine DNA glycosylase repair. PFA – platelet function assay; PWV – pulse wave velocity; RCT – randomised controlled trial; SAE—small airway epithelium; SBP – systolic blood pressure; Stat. sig. diff. – statistically significant difference; TC – tobacco cigarette; TREX1 three prime repair exonuclease 1: VP – vaping product, nnVP – non-nicotine vaping product; TP53 - Tumour suppressor gene; XRCC2-X-ray repair cross complementing 2. Risk of bias for RCTs were assessed using Cochrane RoB2. Longitudinal studies assessed by ROBINS-I tool. Cross sectional studies assessed by Biocross.

Studies in human and animal cells

Study characteristics

Our search identified 11 human cell and one animal cell study (see table 7 in the appendices). Three were funded by the tobacco industry (100, 101, 110) (see funding table 8 in the appendices). One study was conducted in the UK (100), one in Switzerland (101) and the rest in the US. Five studies examined cells from the lung (100, 102-105) 2 studied oral cells (106, 107), one study assessed lung and bladder cells (108); one assessed lung and oral cells (101), one study used epithelial cancer cell lines from the head and neck area (109), one used liver cancer cells and bronchial epithelium cells (110). The animal study involved mouse stem cells (111). Five studies compared cells that had been exposed to vaping product aerosol with cells exposed to tobacco smoke (100, 101, 107, 109, 110) and 7 had either an unexposed or no comparison group (102-108, 112).

Summary of findings

Czekala and colleagues (100) reported that exposure to vaping aerosol, with or without blueberry flavouring had no effect on tissue viability, barrier function or secretion of inflammatory cytokines, oxidative stress, or caused DNA damage in airway cells, while exposure to tobacco cigarettes resulted in significant changes in these outcomes. Iskander and colleagues (101) reported that exposure to vaping triggered alterations in gene expression, but at a lower extent than that observed following exposing buccal and airway cells to tobacco smoke. There was no difference between different vaping product compositions (with or without nicotine or flavourings) though molecular and cellular changes were tissue type-specific. Tellez and colleagues (107) found oral cells exposed to tobacco smoke showed greater signs of toxicity than those exposed to vaping product aerosol. The tobacco smoke condition caused dose-dependent increase in DNA damage whereas, none of the e-liquids delivered in different PG/VG ratios, with or without nicotine caused significance increase in DNA damage. Wieczorek and colleagues (110) reported that tobacco smoke induced a significant and substantial increase in cytotoxicity, mutagenicity and genotoxicity in liver cancer cells and lung cells, whereas cells exposed to vaping product aerosol, there was reduced cytotoxicity compared to cigarette smoke exposure, but no mutagenic nor genotoxic response. Manyanga and others (109) examined the effects of vaping product aerosol compared with unexposed cells or tobacco smoke extracts to see if the aerosol modified cancer response to the chemotherapy drug cisplatin. The authors observed similar impact of aerosol from vaping products and tobacco smoke extracts on cisplatin-induced cell death and cell viability as well as cisplatin resistance and expression levels of DNA repair genes in human epithelial cancer cell lines from different head and neck regions.

The studies that assessed cells exposed to vaping product aerosol with an unexposed group or no group, found DNA damage, reduced DNA repair activity and cell death (see table 7 in the appendices for study characteristics and findings). Escobar and others (113) reported that in bronchial cells exposed to propylene glycol only, glycerol only or a

combination at a high wattage (=85 W), produced an increase in NQO1 expression though there was no significant effect at 40 W. As reported in the chapter on flavours, Noel and colleagues (103) reported that cells exposed to butter-flavoured or cinnamon-flavoured vaping product aerosol under sub-ohm conditions was cytotoxic and altered expression of genes associated with biotransformation, inflammation and oxidative stress. Additionally, increased protein levels of 8-hydroxy-2-deoxyguanosine, an indicator of oxidative DNA damage, was found in cinnamon-flavoured VP aerosol-exposed cells. Lee and colleagues (108) reported that both nicotine and the TSNA NNK induced DNA adducts, reduced DNA repair and enhanced mutational susceptibility. Muthumalage (105), Bahmed (104) and Ji (106) and colleagues reported induced DNA damage and apoptosis following exposure to varied vaping products. In the animal cell study, Zahedi and colleagues (112) treated mouse neural stem cells with menthol and tobacco flavoured e-liquids and suggested that mitochondrial DNA damage could potentially interfere with the mitochondrial life cycle and contribute to dysfunction.

Studies that exposed cells to an aerosol from vaping products suggest potential harm is lower or absent relative to exposure to tobacco smoke, except in one study with the chemotherapy drug cisplatin where both tobacco smoke and vaping product aerosol increased cancer cell resistance to treatment. Those studies that exposed human (or in one case mouse) cells to vaping product aerosol compared to air or with no comparison group suggest cell damage from vaping aerosols including DNA damage, reduced DNA repair activity and in some cases cell death.

Studies in animals

Study characteristics

Our search identified 3 studies on cancer development and progression (114-116) and 3 studies examining vaping product-induced DNA damage in lung (108, 117), heart, bladder (108) and testis (118). Four studies used mice models with the remaining 2 studies conducted in rats, where all animals were subjected to whole body exposure of vaping product aerosol compared with an air control group. See table 6 in the appendices for details on levels of nicotine, puff duration and frequency, which varied across studies.

Summary of findings

Tang and others (114) exposed a group of FVB/N strain of mice to 54 weeks of daily 36mg/mL nicotine, a group exposed to a similar regimen to non-nicotine, and a group exposed only to air. At the end of the exposure period just under a quarter developed lung adenocarcinomas and 57.5% bladder urothelial hyperplasia, whereas 5.6% of mice exposed to filtered air developed one lung adenocarcinoma and none were formed in the group that were exposed to 50% PG/VG without nicotine. Of concern, however, is a small number of animals in the control group (n=18 vs. 40 in the vaping group) and a 4-hour long exposure, which was observed to be the longest time period used in the included animal studies and the purpose of such an exposure protocol is to maximise the negative impact

for the induction of pathological changes. The FVB/N strain of mice is highly susceptible to tumour induction in response to carcinogens, with a high rate of malignant conversion.

A study by Huynh and others (116) found mice receiving tail vein injections of breast cancer cells experienced greater lung colonization and lower tumour cell death than mice not exposed to vaping product aerosol.

Using an immunocompetent mouse model, Pham and others (115) demonstrated that mice exposed to vaping product aerosol for 6 weeks had higher rates of breast cancer growth and metastasis, compared with the control group.

Animal studies were conducted to evaluate carcinogenic risk from vaping product exposure through assessment of DNA methylation biomarkers, an indicator of the overall DNA damage. According to Lee and others (108), 12-week exposure to a nicotine-containing vaping product aerosol significantly increased mutagenic DNA adducts (O6-methyldeoxyguanosines and γ-hydroxy-1,N2-propano-deoxyguanosines) in lung, bladder, and heart tissues of 10 FVB/N mice, compared to air controls. This was accompanied by a marked reduction in DNA-repair activity and repair proteins (XPC and OGG1/2) in the lung only. The interpretation of these findings may be that the endogenous nitrosation of nicotine or of its metabolites can be transformed into carcinogenic NNK, which further induces DNA adducts via its metabolites, leading to DNA damage and tumorigenesis.

Increased cancer risk was also associated with the overproduction of free radicals, especially reactive oxygen species (ROS), following vaping product exposure, leading to a higher susceptibly to DNA damage. Two studies from the same research group reported increased levels of oxidative stress markers (ROS, xanthine oxidase, lipid peroxidation and protein carbonylation) and decreased levels of antioxidant enzymes (catalase, superoxide dismutase, NAD(P)H quinone reductase, UDP-glucuronyl-transferase) in lung and testis of Sprague Dawley rats exposed to a vaping product without nicotine for 4 weeks compared to air controls (117, 118). These detrimental effects were attributed to the release of aldehydes through thermal degradation of e-liquids along with ROS overproduction that can compromise the antioxidant and detoxifying enzymatic mechanisms. However, the use of a low-resistance device resulted in the opposite behaviour with general enzymatic up-regulation in the lung tissue due to higher amount of reactive carbonyl species (117). Pulmonary glutathione reductase appeared to be significantly decreased in a 1.5Ω group and increased in a 0.25Ω group compared to air-controls (117), while no effect was observed in testis from treated animals (118). This is in line with increased levels of glutathione observed in heavy smokers, which is involved in detoxification processes (117). These data suggest that imbalance in the enzymatic antioxidant response was tissue-specific and dependent on the customisation of the device settings. Both studies also demonstrated vaping exposure-related up-regulation of testicular lipoxygenase, a tumour promotion marker, along with an induction of pulmonary and testicular cytochrome P450 (CYP) members, including CYP1A1, CYP1A1/2,

CYP2B1/2 and CYP2E1. CYPs strongly contribute to ROS overproduction and, at the same time, play a key role in the bioactivation of procarcinogens and formation of DNA adducts. In addition, a higher rate of DNA unwinding was observed in white blood cells of vaping product exposed animals (118).

Although these studies point to the potential of vaping product exposure to induce DNA damage, adduct formation and carcinogenicity, none of the above studies included a control group whereby animals were exposed to tobacco smoke. It is therefore difficult to judge the importance of these findings in animal models with the respect to the relative benefits or otherwise of vaping in humans. Future studies may be able to address this issue and investigate the effect of vaping product exposure on cancer initiation and progression.

There is usually a lag time of around 20 years for long term smoking and malignancy development, therefore, animal models, under the correct conditions, can help to reproduce long-term exposure to vaping conditions in a shorter time. Mice remain a preferred rodent model due to their advantages of ease of handling and maintaining, availability of transgenic mice, their use in previous tobacco smoke studies as well as their relatively low cost. Additionally, existing literature on tobacco smoking has shown that the metabolic pathway of nicotine in mice resembled that in humans and their in vivo CYP2A activity seem to be significantly higher than in rats. There are however several caveats which concern the validity of the results from animal studies. The biological aging in mice does not follow the same pattern as it does in humans and depends on the stage of life. It has been suggested that during the adult phase, 2.6 mice days are roughly equivalent to one human year and in the pre-pubertal phase, 3.65 mice days is roughly equivalent to one human year (119); daily vaping for 54 weeks (as in the Tang and others study) would therefore be roughly equivalent to a human vaping high nicotine concentration of e-liquid for a lifetime. Also, although whole body exposure of animals is common in biological research and allows multiple rodents to be exposed simultaneously, reducing variability, the exposure of vaping product aerosol on the whole body of the animal often results in skin and oral exposure due to grooming during and after exposures, introducing other exposure routes. Alternatively, nose-only inhalation provides targeted delivery of vaping aerosol to the respiratory system only in order to more closely mimic human exposure to vaping, avoiding the variable dosage from ingestion. The greatest concern, however, is repeated restraint inside a tube and has been previously associated with stress, thermoregulation constraints as well as attenuated body weight gain.

9.4 Conclusions

In this chapter we reviewed the existing evidence on how vaping might affect cancer risk. This included summarising previous reports that have addressed this issue, and then presented findings from our systematic review of health risks and effects of vaping that are relevant to cancer.

Our 2018 evidence review of vaping commissioned by Public Health England (PHE), the report from the US National Academies of Science, Engineering and Medicine in the US and the UK Committee on Toxicity report (27) include some earlier evidence. The previous PHE report included one study directly relevant to cancer that suggested people who switched from smoking to vaping were exposed to lower levels of toxicants and carcinogens than in smoking, but also pointed to the need for further research. The 2018 NASEM report found no clear evidence about whether the chemicals in vaping aerosols or vaping behaviour were associated with cancer risk. The Committee on Toxicity also reported that exiting evidence was insufficient to draw conclusions about any links between vaping and cancer risk in humans.

We identified a growing albeit still modest literature on how vaping may affect cancer risks in humans. In our review of human studies, biomarkers of exposure to several human carcinogens well known in tobacco smoke show clear patterns of differential exposure with lower measured levels in people who vape compared with those who smoke. Hence, the biomarker of exposure studies compiled in this review provide conclusive evidence that the differential in emissions is maintained, at least in a binary sense, and that vaping generally leads to lower exposure to many of the carcinogens responsible for the considerable health risks of smoking. Findings from studies of inflammation and oxidative stress do not demonstrate any systematic relationship with mixed evidence of differences (or no difference) in levels between vapers and smokers and non-users, this evidence is currently insufficient to draw conclusions.

We identified 2 RCTs, one longitudinal study and 5 cross sectional studies of gene expression and DNA methylation in humans. Methodological limitations (for example, lack of smoking comparison groups in some studies) constrain what we can say about these epigenetic studies, as there is currently insufficient data, nevertheless methylation and demethylation of specific genes related to smoking and vaping show potential for shedding light in this area.

There were no studies that assessed how vaping affects people with an existing or prior cancer condition.

It is challenging to directly translate the findings from pre-clinical studies using human or animal cells or rodent models to any cancer risks arising from vaping in humans. These pre-clinical studies commonly employ acute exposures sometimes over concentrated periods, and it is unclear whether the mechanisms or pathways to risk identified would be replicated in vapers. Further challenges arise because of the complex nature of vaping behaviour over time and the wide variety of different aerosols and products used. Despite these significant limitations, there are indications from this literature that vaping is not benign to people who have never smoked, and that exposure may lead to the identification of biomarkers of harm or be implicated in negative outcomes that may affect the viability of cancer treatment for those with pre-existing disease. However, cell and animal studies

appear to support the human studies and suggest vaping may trigger alterations in gene expression, but at a lower extent than that observed following exposure to tobacco smoke.

9.5 Implications

Although vaping generally leads to lower exposure to many of the carcinogens responsible for the considerable health risks of smoking, studies of biomarkers of exposure that are associated with cancer risk in humans need to have longer follow up periods than has been the case to date.

More research is needed on biomarkers of potential harm in humans.

Studies applying potentially important novel methods to assess vaping often neglect to include cigarette smoke as a comparator as well as a control (usually filtered air). Even when a tobacco smoke comparison group is included it is often difficult to compare like with like when the exposure to nicotine and other important parameters are not included in the description of the experiments. Such data are essential when assessing whether human exposure to different forms of nicotine delivery, in this case vaping and smoking, result in different magnitudes of cancer risk.

Further studies are needed to identify the extent to which evidence from pre-clinical studies is directly relevant in humans.

There are a number of gaps in the literature identified in our review also including some gaps that came to our attention when preparing the background to this chapter. Although much is known about the links between tobacco smoking and cancer, more needs to be done to document the smoking status of cancer survivors, who will make up an increasing proportion of cancer patients in the future given improvements in survival and an ageing population, which means that the risk of recurrence or a secondary cancer will not be uncommon.

We could not identify any studies from the UK on vaping prevalence among people diagnosed with cancer or cancer survivors, so this should be a further area of research.

In addition, more research is needed with cancer patients and cancer survivors to understand any role for vaping as a smoking cessation aid in improving treatment outcomes or reducing the risk of cancer recurrence.

Studies are also needed that assess the effects of vaping on cancer outcomes in people diagnosed with cancer, both in comparison with no use of nicotine or tobacco products and in comparison with smoking.

For policy makers and practitioners, findings from our review for this chapter suggest that developing and implementing policies and interventions that support smokers to completely switch from smoking to vaping will reduce exposure to toxicants and carcinogens which may have relevant outcomes for cancer prevention.

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10 Respiratory diseases

10.1 Introduction

Smoking and respiratory diseases

This chapter focuses on respiratory diseases, one of the main causes of smoking-caused premature mortality and morbidity. It describes the relationship between smoking and respiratory diseases, then describes how vaping might cause respiratory diseases and then summarises findings from previous reports. The chapter then gives an overview of studies identified in our systematic review which present evidence on associations between vaping and respiratory health.

The main respiratory disease caused by smoking is chronic obstructive pulmonary disease (COPD), a progressively debilitating disease which encompasses chronic bronchitis (long-term inflammation of the airways) and emphysema (damage to the alveoli) (1). Tobacco smoke inhalation is the most common risk factor for COPD and related deaths (2, 3), and COPD is the third leading cause of death worldwide, causing over 3 million deaths in 2019 (4). Smoking also increases the risk of other respiratory diseases such as pneumonia (5), invasive pneumococcal disease (6), sleep apnoea and tuberculosis (7). Smoking also exacerbates asthma (7) and increases the risk of influenza 5-fold (5). Respiratory conditions can be further compounded by the association between chronic respiratory and cardiovascular disease (CVD) (8). People with COPD have an increased risk of CVD and up to one third of people with COPD die from CVD (9). Passive smoking also causes respiratory disease in non-users, including effects in utero, effects on children and respiratory diseases in adults (7, 10). The role of smoking and passive smoking on lung cancer is covered in chapter 7 (cancer).

In England, of the 74,600 deaths attributable to smoking in 2019, 23,700 (32%) were respiratory diseases and in that year, just over a third (35%) of all deaths from respiratory diseases were attributable to smoking (11). Stopping smoking or never smoking are the most effective ways of preventing respiratory disease. For people with COPD, stopping smoking does not reverse the underlying lung damage but can help to reduce the severity of COPD symptoms and decelerate the speed with which COPD worsens (1, 12). Studies have shown that after 5 years since stopping smoking, the risk of acquiring pneumonia reduces by about half (13) and stopping smoking can reverse worsening asthma symptoms and lung function (14).

How smoking causes respiratory disease

Most people who vape have a history of tobacco smoking, so we summarise here how tobacco smoking affects respiratory disease. As tobacco smoke is inhaled, it damages the airways and the lungs, including the alveoli which are the small air sacs in the lungs. For example, inhaled particulate matter from cigarette smoke is deposited in the airways, with larger particulates being deposited in the upper airways and smaller particulates in the alveoli.

Findings of a US Food and Drug Administration sponsored workshop on biomarkers of potential harm (BoPH) associated with tobacco and nicotine products (15) identified several mechanisms through which tobacco smoke constituents could damage the lung. The authors commented that many of the same constituents of tobacco smoke can cause different diseases, noting for example that smoking causes oxidative stress and inflammation which alongside infection and other processes can play a role in the pathways to CVD, COPD and cancer. In relation specifically to mechanisms of lung damage, the workshop authors noted that tobacco smoke constituents could: impair the lung's innate defence or immune system, leading to the potential for infection and inflammation (for example from acrolein); be toxic to the cilia or microscopic hairs along air passages (for example from acrolein and formaldehyde); irritate the lung (for example from formaldehyde); cause oxidative damage (for example from nitrogen oxides, cadmium) and disrupt the oxidative metabolism of cells (for example from hydrogen cyanide).

Oxidative stress can lead to chronic inflammation which plays a key role in lung disease and pro-inflammatory effects on the lung can be observed before diseases are diagnosed. A recent review (16) indicated the multifaceted processes specifically involved in COPD including oxidative stress, inflammation, proteinase/anti-proteinase imbalance, tissue destruction and inadequate repair. For example, tobacco smoke affects proteolysis which involves the degradation of proteins; proteases and antiproteases are produced by the respiratory epithelium and the balance of these are important for respiratory homeostasis. The review highlighted the role that alveolar macrophages may play in these processes. The review indicated that alveolar macrophages act as gatekeepers 'exerting regulatory effects such as phagocytosis, the production of inflammatory mediators such as ROS and the expression of inflammatory cytokines such as IL-1, IL-2, IL-4, IL-6, IL-8, TNF-α and interferon gamma'. As well as initiating immune responses, alveolar macrophages also resolve them through release of anti-inflammatory mediators and clearance of apoptopic bodies (efferocytosis). They also play a role in lipid processing and iron homeostasis.

How vaping might affect respiratory risks

Turning to how vaping might affect respiratory risks and given damage to the respiratory system caused by smoking might persist, it is important to note that the effect of vaping may differ dependent on whether airways are already damaged by smoking or are healthy.

The National Academies of Sciences, Engineering and Medicine (NASEM) (17) suggested several possible biological pathways for how vaping may theoretically influence respiratory disease, some of which may be similar to smoking, for example how smoking affected several host defence mechanisms in the lungs. NASEM specifically indicated that any ultrafine particle exposure could damage airways and lung parenchyma, through damage to the DNA, the induction of pro-inflammatory cytokine expression, adverse effects on the immune system through the production of free oxygen radicals; ultrafine particle exposure could also increase the rate of asthma exacerbations. Inflammation has been noted as potentially an important pathway between vaping and respiratory risks (18). In chapter 5 (nicotine) we indicated that NASEM identified 3 putative pathways through which nicotine could damage the respiratory system or worsen pre-existing lung disease: decreased viral and bacterial clearance; impaired cough; and alpha7 nicotinic acetylcholine receptor activity and cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction in the airways. Also indicated in chapter 5 (nicotine) NASEM suggested that flavourings could also alter cellular redox balances in the airways by increasing pro-inflammatory cytokines. While aversive to vapers, high temperatures cause the production of formaldehyde, which would lead to toxic effects on the lungs. NASEM indicated that the impact of passive vape exposure also needed to be examined.

Detecting respiratory diseases

As most people who vape have a history of smoking, many vapers are likely to have either existing respiratory disease or have an increased risk of respiratory disease, and it is important to separate the impact of prior smoking from vaping, and/or whether vaping could alter the progression of pre-existing respiratory disease. There are several tests for respiratory disease which are summarised here. These informed the scope of the studies included in our review; biomarkers of potential harm were largely informed by the FDA sponsored workshop referred to above (15) and excluded the subjective self-reported symptom measures described below as explained in chapter 2 (methods).

Spirometry and other breath and lung function tests

Spirometry is a breath test that assesses the presence of airflow obstruction and is commonly used to detect respiratory diseases including COPD and asthma. Indeed, COPD has been identified by spirometry in people who had not yet had a diagnosis of respiratory disease (19), suggesting under-diagnosis is common. The most common measures of lung function measured by spirometry include forced expiratory volume in one second (FEV1), forced vital capacity (FVC), the FEV1/FVC ratio, peak expiratory flow (PEF) and forced expiratory flow (FEF) 25-75%. FEV1 is strongly correlated with COPD disease severity but is not considered to be highly sensitive to lung disease (15). FEV1 is relatively stable from 20 to 35 years of age and then declines, but more rapidly in people with COPD (20), although the decline in FEV1 slows down after stopping smoking. FVC reflects impaired lung development, accelerated loss of lung units and the presence of gas

trapping due to emphysema and airway collapse. FEV1/FVC ratio reflects the presence of airflow obstruction. PEF is how quickly a person can breathe out after a full inhalation and is frequently used for diagnosing and monitoring asthma. FEF was not highlighted in the FDA workshop so is not covered in our review.

Fractional exhaled nitric oxide (FeNO, a measure of how much nitric oxide is in the breath) is elevated by asthma as it is a marker of airways inflammation. FeNO is therefore a biomarker of asthma (20). Smoking reduces FeNO, but FeNO has been shown to differentiate asthmatic subjects from non-asthmatic subjects both in smoking and never smoking groups in a population with asthma-like symptoms (21). Complete abstinence from smoking for 41 weeks has been observed to lead to near normalisation of FeNO levels in a sample of smokers in good general health with no doctor-diagnosed respiratory diseases (22). NASEM noted other measures can be used to assess respiratory diseases: residual volume increases, detected through body plethysmography, which can correlate with worsening airflow obstruction; and impulse oscillometry which detects changes in large and small airway resistance. Impulse oscillometry is non-invasive and uses sound waves to allow passive measurement of lung mechanics. It has been used to diagnose and monitor asthma, and potentially early stages of COPD. A loudspeaker at the mouth generates an impulse (mixture of sound waves of all frequencies typically from 5 to 30 hertz (Hz)) into the lungs which causes changes in pressure and air flow in different parts of the airways. These are measured though attachments to the mouthpiece, which give data on respiratory impedance (which comprises resistance and reactance), resonant frequency, area of reactance and coherence. NASEM noted that these might be more sensitive than spirometry in detecting reversibility of airway obstruction for people with COPD, but it is not yet used in standard clinical practice other than where spirometry is more difficult such as in younger or older patients.

As the lungs facilitate gaseous exchange with the circulatory system, changes in this can be assessed with proton magnetic resonance imaging (MRI) using the ratio of ventilation (flow or air into and out of alveoli) to perfusion (flow of blood to alveolar capillaries), commonly referred to as V/Q.

Other respiratory biomarkers

The US Food and Drug Administration sponsored workshop mentioned above (15) also identified imaging biomarkers such as computed tomography (CT) scanning and MRI to detect structural changes in the lung, which also may have greater sensitivity than FEV1 for detecting emphysema and lung disease. NASEM also indicated that ultra-low-dose CT and MRI might be alternative modalities to conventional chest CT in assessing COPD changes (17). Positron emission topography or PET scans could also be used to provide detailed 3-dimensional images either separately or in conjunction with CT or MRI scans.

Biomarkers of inflammation assessed directly in the lung could also be useful indicators of lung disease, such as neutrophils measured in samples from bronchoalveolar lavage bronchoscopies (BALs), and pro-inflammatory cytokines from bronchial biopsies (18). Chang and others (15) also identified persistent systemic inflammation based on white blood cell count, CRP, IL-6, TNF-alpha, fibrinogen and IL-8 as associated with COPD outcomes, and soluble receptor for advanced glycation end products (sRAGE) as a useful indicator of severity of emphysema and disease progression as well as CFTR ion channel activity. Chang and others (15) noted the relative amount of M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages had been shown to differ between non-symptomatic smokers and non-smokers. Biomarkers of oxidative stress such as 8-isoprostane can also be informative for assessing respiratory disease.

Chang and others (15) also noted the potential of ‘omics’ biomarkers to provide information on mode of action and dose-response relationships.

Biomarkers of exposure (23) that may be relevant to respiratory diseases include nicotine, carbon monoxide, volatile organic compounds, specifically acrolein, acrylonitrile, 1,3-Butadiene, acetaldehyde, formaldehyde, toluene, polyaromatic hydrocarbons, acetone, ammonia, m-Cresol, o-Cresol, p-Cresol, hydrogen cyanide, nitric oxides and phenol.

Respiratory symptoms

Although out of scope in our review, NASEM also commented that standardised respiratory questionnaires could be helpful in evaluating outcomes. Cough reflex sensitivity, urge to cough and nasal mucociliary clearance are defence mechanisms which help clear pathogens and pollutants from the lungs and sinuses. Additionally, self-reported wheeze, shortness of breath, mucus/sputum production, other respiratory symptoms and quality of life could also be helpful to assess. The St George’s Respiratory Questionnaire (SGRQ) is a disease specific quality of life measurement which is validated for both COPD and asthma. Additionally, the COPD Assessment Test (CAT) is self-administered and measures the impact of COPD on health-related quality of life. As self-reported symptoms were outside the scope of our review these are not covered, but we have included these for the smaller number of studies included in our systematic review which focused on participants with existing disease, for example, people suffering from COPD or asthma, where appropriate.

Measures of exercise capacity, which integrate respiratory, cardiac and skeletal muscle function as well as parameters from cardiorespiratory exercise testing are also used to assess the impact of respiratory disease and may be abnormal even in ostensibly healthy smokers (24). Sleep parameters might also be affected. These are however outside the scope of our review, although again included where relevant for the small number of studies on participants with existing disease.

Finally, given the strong relationship between respiratory and CVD and deaths from CVD, biomarkers of cardiovascular disease can also be used to predict all-cause mortality from COPD (9).

10.2 Previous reports about the effects of vaping on respiratory disease

Overview

Previous comprehensive reports on the effects of vaping on health come from NASEM in the US and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in the UK, published in 2020 (17, 25). COT is an independent scientific committee that provides advice to the Food Standards Agency, the Department of Health and other government bodies on matters concerning the toxicity of chemicals. COT is an advisory non-departmental public body.

In the Methods chapter (chapter 2) we explain the rationale for summarising these reports. The summary of reports and our systematic review below include human, cell, and animal studies. We give priority and most weight to human studies. We include findings from cell and animal studies for completeness but noted in chapter 2 (methods) their limitations and lack of transferability to humans, and comment on any specific notable limitations for individual studies in the narrative below.

Previous evidence reviews on vaping, commissioned by Public Health England (PHE)

In our previous 2018 report (26) (which was not a systematic review but included literature until mid-August 2017) we identified 4 small or uncontrolled studies suggesting some benefits to respiratory health when smokers switched to vaping. Two further studies of adolescents suggested that there was an association between self-reported respiratory symptoms and trying vaping, but both suffered from confounding. Our 2018 report also examined studies of biomarkers of exposure. Eight papers covering 7 studies with 658 participants assessed the biomarker 3-HPMA, a biomarker of the volatile organic compound (VOC) acrolein which is a potent respiratory irritant. Levels were about 60% lower in vapers compared to cigarette smokers. Nine papers reported on 8 studies with 245 participants of carbon monoxide (CO), finding levels to be about 78% lower than in cigarette smokers. In studies that compared 3-HPMA and CO between vapers and non-users, levels were comparable.

The National Academies of Sciences, Engineering and Medicine report on the Public Health Consequences for E-Cigarettes

The NASEM report (17) (which searched the literature to the end of August 2017) included 17 human studies (3 of which were covered in the 2018 PHE report (26) above) that examined respiratory outcomes in people who used vaping products. Studies included people switching to vaping products either exclusively or alongside cigarette smoking and included smokers with or without respiratory diseases. NASEM concluded that most of these studies provided support for beneficial health effects relative to continued use of tobacco smoking, but the one randomised control trial (RCT) included in the review, found no improvements in lung function after 12 weeks for those that switched (27). NASEM indicated that as the majority of these studies had small sample sizes and involved subjects selected retrospectively and combined with the fact that 6 of them emanated from one group of researchers in Italy, NASEM concluded that there was limited generalisation of the results.

NASEM identified 2 studies examining short-term effects of vaping products on respiratory health (among smokers as well as participants without a history of smoking) and concluded that nicotine-containing vaping products but not non-nicotine vaping products could have short-term adverse effects on lung defence mechanisms such as mucociliary clearance, urge to cough and cough sensitivity.

NASEM noted that there was a lack of well-designed epidemiological studies examining the long-term effects of vaping product use on the development of chronic respiratory symptoms on smokers with or without respiratory disease. NASEM also noted that there was a lack of rigorously designed epidemiological studies examining the relationship between chronic vaping product use in adolescents and young adults and respiratory disease and no epidemiological studies examining respiratory effects of passive exposure to vaping.

Overall, when including findings from animal and cell studies NASEM concluded that there was:

- no available evidence whether or not e-cigarettes cause respiratory disease in humans
- limited evidence for improvements in lung function and respiratory symptoms among adult smokers with asthma who switch to e-cigarettes completely or in part (dual use)
- limited evidence for reduction of COPD exacerbations among adult smokers with COPD who switch to e-cigarettes completely or in part (dual use)
- moderate evidence for increased cough and wheeze in adolescents who use e-cigarettes and an association with e-cigarette use and an increase in asthma exacerbations

- limited evidence of adverse effects of e-cigarette exposure on the respiratory system from animal and in vitro studies

The Committee on Toxicity (COT) Statement on the potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems

COT did not identify data about repeated or long-term inhalation exposure to nicotine in humans (separate from tobacco smoke) or any data on long term effects of nicotine exposure from vaping products (see chapter 5 on nicotine). COT noted nicotine had adverse effects on human respiratory systems including cough suppression and mucociliary clearance, and adverse effects on animal respiratory systems namely increased airways hyper-responsiveness and impaired mucociliary clearance, and the development of respiratory systems (25). COT also noted potential respiratory impacts of certain flavours, such as menthol, vanillin and cinnamaldehyde (chapter 6 on flavours). COT noted particular concerns for naïve users of vaping products who had respiratory sensitivity such as those with COPD, asthma and cystic fibrosis.

10.3 Findings from the systematic review

Overview

As outlined in chapter 2, our systematic review addressed 2 aims:

1. What effect does vaping and second-hand exposure to vaping products have on biomarkers that are associated with the risk of respiratory diseases?

2. What are the effects of vaping among people with existing respiratory diseases on disease outcomes?

The methods for the systematic review are explained in chapter 2. Quality assessments and the funding source of each study can be found in the appendices.

The remainder of this chapter will provide a summary of studies in humans that assessed biomarkers of exposure with relevance to respiratory disease, which were presented more fully in chapter 6. It will then summarise findings for biomarkers of potential harm associated with respiratory diseases which also cut across several diseases, specifically biomarkers of oxidative stress, inflammation and endothelial harm, again from studies in humans, which were presented more fully in chapter 7. This will be followed by a summary of studies looking at additional biomarkers of respiratory diseases, such as spirometry measures. Finally, findings from in vitro (cell) studies and in vivo (animal) studies with relevance to respiratory diseases will be summarised.

Biomarkers of exposure with relevance to respiratory disease

In chapter 7, we report findings on biomarkers of exposure in detail. Here, we summarise the findings from biomarkers of toxicants which have relevance to respiratory diseases: nicotine, carbon monoxide, volatile organic compounds, (specifically acrolein, acrylonitrile, 1,3-Butadiene, formaldehyde and toluene) polyaromatic hydrocarbons, hydrogen cyanide and o-Cresol. Other toxicants that affect respiratory disease such as acetone, ammonia, m-Cresol, p-Cresol, phenol and acetaldehyde, known to be associated with tobacco smoke, were not included in any of the studies identified in the systematic review.

Nicotine

We identified 60 studies, including 5 meta-analyses of nicotine and nicotine metabolites among at least weekly vapers and smokers. The evidence from these studies suggested that over time and with increased experience of vaping, users can derive similar levels of nicotine as they can from smoking cigarettes. Levels of nicotine metabolites also varied with vaping frequency, length of vaping and type of vaping products used.

Carbon monoxide

Thirty-two studies identified in the systematic review assessed exposure to expired air carbon monoxide (CO) and/or blood carboxyhaemoglobin (COHb). One RCT found CO levels had statistically significantly decreased by 84.4% compared with baseline, after completely switching from smoking to vaping for 7 days; this was similar to significant CO reductions among those assigned to non-use (85.7%) (28). Average changes in CO exposure in the 3 other RCTs were likely dependent on adherence to the vaping only condition and found statistically significant (29) and non-significant reductions (31, 180) in CO in the short and long term. A meta-analysis of findings from 2 RCTs found the average geometric mean of COHb were approximately 76% lower among vapers than among smokers, which was statistically significantly with no heterogeneity between studies (32, 33). Another RCT (34) reported statistically significant average reductions in COHb levels in groups assigned to vaping (58%), nicotine gum or lozenge users (45%) and dual users (22%) 8 weeks after switching from exclusive smoking (34).

We also meta-analysed 4 acute cross-over studies of expired CO levels (30, 35-37) and separately, a further 3 longitudinal studies (38-40). In both meta-analyses, we found a statistically significant reduction in expired air CO in vaping groups compared with smoking groups, with a geometric mean approximately 63% lower among vapers than smokers in both studies. There was considerable heterogeneity between the studies, but the direction of the difference was consistent across reported findings in both meta-analyses. A further cross-over study reported statistically significant reductions in expired air CO levels during vaping (reduction of ~40.8%) and non-use (reduction of ~26.0%) conditions though some participants continued to smoke during the vaping condition (41). Another cross-over study did not find a statistically significant difference in CO levels between acute exposure to

vaping or a sham vaping condition (42). Reduction in expired air CO levels was reported in 8 other longitudinal studies where smokers switched to vaping from smoking for 2 weeks to 6 months (43-48), and 2 studies where participants reduced the number of tobacco cigarettes smoked per day following assignment to vaping groups (49, 50). In one long term study following up daily ad libitum vapers who had never smoked for 42 months, reported median CO levels were similar between vapers and non-users' groups (51). A study that switched vapers who were ex-smokers to a heavy smoking condition reported a statistically significant over 6-fold increase in expired air CO levels after a week of smoking and a statistically significant 2-fold increase in blood COHb levels (52). A further study reported a statistically significant reduction in blood COHb levels by over 80% 6 months after switching from smoking to vaping (48).

Two studies compared expired air CO levels between smokers and vapers, reporting levels to be on average between 47% (53) and 75% (54) lower among vapers compared to smokers, both differences were statistically significant.

Volatile organic compounds (VOCs)

Twenty-four studies assessed exposure to VOCs, with considerable variation across the studies in terms of design, definitions of vaping and smoking, biomarker measurements and exposure duration. To assess relative exposures between vaping and smoking, we carried out 15 meta-analyses of results from studies that measured VOCs. In general, most showed statistically significantly lower levels of VOCs among vapers than smokers, with substantial reductions in some biomarkers.

Acrolein

All 24 studies assessed acrolein, which is a respiratory irritant, and its main urinary metabolites 3-hydroxypropylmercapturic acid (3-HPMA) and N-acetyl-S-(carboxyethyl)-l-cysteine (CEMA). We meta-analysed 3 RCTs (32-34) that measured 3-HPMA and found a pooled geometric mean 3-HPMA level 71% lower among vapers than among smokers' average levels, which was statistically significant; there was considerable heterogeneity, but the direction of difference was consistent across studies. In a separate meta-analysis, findings from 2 longitudinal studies (39, 55) were pooled and also showed statistically significantly lower average levels of 3-HPMA in vapers than smokers, with no heterogeneity. Pooling data from 3 cross-sectional studies (56-58) that measured 3-HPMA supported the estimates from the RCT and longitudinal meta-analyses; the pooled geometric mean 3-HPMA level was 45% lower among vapers compared to smokers, with moderate heterogeneity.

In the studies that were narratively synthesised and not included in a meta-analysis, one RCT and one cross-over study reported significantly lower 3-HPMA levels in vapers than smokers (28, 59). Two longitudinal studies found no significant difference in 3-HPMA levels between vaping and smoking groups, though some vapers may have been smoking

in these studies (44, 60). One longitudinal study (61) found no statistically significant changes in urinary 3-HPMA levels 6 weeks after smokers who were diagnosed with mental health disorders had been encouraged to use vaping products instead of cigarettes; nearly all participants at the follow-up were ‘dual users’.

Two of the RCTs and one longitudinal study reported statistically significant reductions in levels of urinary CEMA in participants in vaping groups compared to those in smoking groups (28, 34, 55). Six cross-sectional studies reported CEMA levels to be statistically significantly different, between approximately 26% (58) and 98% studies (62) lower among vapers compared to smokers. A meta-analysis of 3 cross-sectional studies did not find a statistically significant difference for CEMA levels between vapers and smokers (56-58).

Seven cross-sectional studies (56-58, 62-65) reported levels of 3-HPMA among vapers and non-users and meta-analysis of 3 studies found 3-HPMA levels were not statistically significantly different in vapers compared with non-users (56-58). Nine cross-sectional studies (56-58, 62-64, 66, 67) reported levels of CEMA among vapers and non-users, 3 of which were combined, and their pooled geometric mean was 12% lower among vapers than non-users, though this was not statistically significantly different.

Across cross-sectional studies that measured urinary biomarkers of acrolein, vapers’ CEMA levels were approximately between 2% and 74%, and non-users’ levels were approximately between 1% and 81%, of CEMA levels among smokers. Across studies that measured urinary 3-HPMA, vapers’ levels were approximately between 17% and 62%, and non-users’ levels were between 12% and 68%, of 3-HPMA levels detected among smokers (chapter 7, figure 21).

Acrylonitrile (CNEMA)

Sixteen of the 24 studies assessed levels of acrylonitrile, a respiratory toxicant and its main urinary metabolite 2-cyanoethyl mercapturic acid (CNEMA). One RCT, one cross-over study and 2 longitudinal studies found acute or short term statistically significant reductions of 80-90% in smokers who switched to vaping (33, 39, 44, 59). A meta-analysis of 3 cross-sectional studies found a pooled geometric mean urinary CNEMA level 94% lower among vapers compared to smokers (56-58). A further 6 cross-sectional studies not included in the meta-analysis also found statistically significant lower levels of CNEMA in vapers compared with smokers (75% to 99% lower) (57, 58, 62, 64, 67, 68) and a further 2 studies found 88% to 93% lower levels though these were not tested for statistical significance (56, 69). Compared to non-use, urinary CNEMA levels were around 3 times higher after vaping product use and 4 times higher after smoking, suggesting that vaping might increase exposure to acrylonitrile in absolute terms (55).

Across cross-sectional studies that measured urinary acrylonitrile metabolite CNEMA, vapers’ levels were approximately between 0.2% and 22.6%, and non-users’ levels were

approximately between 0.2% and 7.5%, of CNEMA levels among smokers (chapter 7, figure 24).

1,3-Butadiene (MHBMA, DHBMA)

Eleven of the 24 studies identified in the systematic review assessed levels of 1,3-Butadiene and its 2 urinary biomarkers, monohydroxybutenyl mercapturic acid (MHBMA) and dihydroxybutylmercapturic acid (DHBMA).

Three RCTs found significantly lower levels of MHBMA in vapers compared with smokers (28, 32, 33). A meta-analysis of 2 RCTs (32, 33) found that the geometric mean MHBMA levels were approximately 83% lower among vapers than among smokers. A cross-over (59) and 2 longitudinal studies (39, 55) also found significantly lower levels in vapers than smokers. Pooling 2 studies found geometric mean MHBMA levels were approximately 99% lower among vapers than among smokers (39, 55). Six cross-sectional studies supported these findings; when combining 3 of these studies, geometric mean urinary MHBMA levels were 84% lower among vapers compared to smokers, though heterogeneity was substantial (56-58).

Seven cross-sectional studies reported levels of MHBMA among vapers and non-users (56-58, 62-65). Levels from studies among adults were reported to range from around 42% lower (64) among vapers compared to ex-smokers who use nicotine replacement therapy (NRT), to 104% higher (62) among vapers compared to non-users, and differences were not statistically significant. A further study reported levels to be 14% lower among vapers compared to non-users, however this was not tested for statistical significance (56). One study reported a statistically significant difference between adolescent vapers and non-users, even though the groups reported the same median levels of MHBMA (65). Combining 3 studies, the pooled geometric mean urinary MHBMA level was 4% lower among vapers compared to non-users, which was not statistically significant (56-58).

Across cross-sectional studies that reported urinary MHBMA, vapers' levels were approximately between 13% and 35% and non-users' levels were between 7% and 32% of levels detected among smokers (chapter 7, figure 34).

One longitudinal study reported that levels of urinary DHBMA among vapers did not differ from levels among smokers, tobacco pouch users or non-users (55). Four cross-sectional studies reported DHBMA to be statistically significantly lower among vapers compared to smokers, by between approximately 23% (64) and 45% (62). One study reported levels to be on average 33% lower among vapers compared to smokers, however this was not tested for statistical significance (56). One study reported levels to be statistically significantly higher, by 11%, among vapers compared to smokers (58). Combining 3 cross-sectional studies (56-58), the pooled geometric mean urinary DHBMA level was 14% lower among vapers compared to smokers which was not statistically significant.

Across cross-sectional studies that measured urinary DHBMA, vapers' levels were approximately between 56% and 111% and non-users' levels were approximately between 52% and 115% relative to urinary DHBMA levels detected among smokers (chapter 7, figure 34).

Formaldehyde (formate)

Two of the 24 studies assessed urinary levels of formate. Levels did not change after a single use of a vaping product or smoking (55). Urinary formate levels were higher after a week of using a vaping product with 6 milligrams per millilitre (mg/mL) nicotine with an adjustable-powered device than after using 18mg/mL nicotine with the same type of device (70). The authors suggested formaldehyde exposure might increase during compensatory puffing behaviour with lower nicotine strength e-liquids. No cross-sectional studies reported levels of formate.

Toluene (S-BMA)

Five of the 24 studies reported on toluene and its main metabolite S-benzylmercapturic acid (S-BMA). One longitudinal study reported average S-BMA levels did not differ between non-users and after a single use of a vaping product, tobacco cigarette or a tobacco pouch (55). Four cross-sectional studies (56, 58, 62, 63) reported levels to be between 4% lower (62) and 28% higher (58) among vapers compared to smokers, however neither comparison was statistically significant. One study reported levels were 3% higher among vapers compared to smokers, however this was not tested for statistical significance (56). In a meta-analysis of 2 studies, the pooled geometric mean urinary S-BMA level was 19% higher among vapers compared to smokers and the difference was not statistically significant (56, 58). Levels were either 36% statistically significantly lower than in vaping groups compared with non-users (58, 62), statistically significantly higher by an average 11% among vapers compared to non-users in one study (39) and no different between vaping and non-using groups in another (56). Combining 2 cross sectional studies, the pooled geometric mean urinary S-BMA level was 21% lower among vapers compared to non-users though the difference was not statistically significant, with substantial heterogeneity between studies (56, 58).

Across cross-sectional studies that measured urinary toluene biomarker S-BMA, vapers' levels were approximately between 97% and 128% and non-users' levels were approximately between 101% and 200% of S-BMA levels detected among smokers (chapter 7, figure 42).

Polyaromatic hydrocarbons (Benzo[a]pyrene (3-OH-B[a]P) & pyrene (1-HOP))

Based on findings from 2 RCTs, one cross-over and one longitudinal study, exposure to polyaromatic hydrocarbons appears to be reduced significantly, by around 60%, after switching from smoking to vaping product use for at least 5 days (28, 33, 41). One RCT reported lower exposure to polyaromatic hydrocarbons among nonusers compared with

vapers 7 days after randomisation (28). Overall, cross-sectional studies suggest that urinary 1-HOP is significantly lower among vapers compared to smokers (63, 67, 69, 71). Levels were reported to be higher among vapers compared to non-users (63, 67, 71).

Across cross-sectional studies that measured urinary 1-HOP, vapers' levels were approximately between 52% and 67% and non-users' levels were approximately between 33% and 44% of 1-HOP levels detected among smokers (chapter 7, figure 52).

Other biomarkers of exposure

Hydrogen cyanide (thiocyanate) and Ortho-Cresol (o-Cresol)

One RCT reported statistically significant reductions in thiocyanate levels by around 36-39% after 5 days of switching from smoking to vaping a cartridge vaping product with 48mg/mL nicotine and statistically significant reductions in thiocyanate levels by approximately 29% in smokers who switched to use of 4 mg nicotine gum (33). In another publication of the same RCT, statistically significant reductions in both urinary and blood plasma o-Cresol sulfate levels were reported by around 80% compared with baseline after switching from smoking to vaping (72). No cross-sectional studies reported levels of hydrogen cyanide or o-Cresol.

Summary of biomarkers of exposure to potential respiratory toxins

Studies indicated substantially lower levels of carbon monoxide exposure among vapers than smokers, and some interventional studies suggested exposure to carbon monoxide in smokers who completely switch to vaping might be reduced to levels similar to non-users. Thus, carbon monoxide exposure would not appear to be a factor in respiratory disease risk among vapers. Evidence suggests that with time and experience users can derive similar levels of nicotine to from smoking cigarettes. Thus, given NASEM and COT speculated that putative pathways to respiratory disease could involve nicotine, the potential exists for nicotine vaping to contribute to respiratory risks from vaping. Findings for nicotine and carbon monoxide are consistent with studies reported in our 2018 evidence review (26).

In general, studies showed statistically significant lower levels of VOCs among vapers than smokers, with the most substantive reductions being observed for the respiratory irritants acrolein (consistent with findings from our 2017 report) and acrylonitrile (CNEMA) and 1,3-butadiene (MHBMA) which had not been summarised previously. For a few VOCs, such as formaldehyde and toluene, available evidence was inconclusive regarding significant differences between vapers and smokers, although one study suggested formaldehyde exposure might increase during compensatory puffing behaviour with lower nicotine strength e-liquids. Cross-sectional research also suggests little difference between smokers and non-users in toluene.

Generally, there were no significant differences between vapers and non-users except for the acrylonitrile metabolite CNEMA for which the evidence suggested that vaping might increase exposure to acrylonitrile in absolute terms.

Biomarkers of potential harm to health cutting across several diseases

In chapter 8, we report findings on biomarkers of potential harm that cut across several diseases in detail. Here, we summarise those findings from cross-cutting biomarkers of potential harm which have relevance to respiratory diseases: 8-isoprostane, white blood cell count, CRP, IL-6, IL-8, TNF-α levels and fibrinogen.

8-isoprostane

Nine studies were identified in our systematic review which assessed 8-isoprostane, one of which also included smokers with asthma.

Four cross-over studies compared changes in 8-isoprostane levels in blood after a single use session (73, 74), 48 hours (75) and 5 days (30) of vaping, dual use, smoking or non-use conditions. One study found statistically significant increases in 8-isoprostane levels after a single use session of a cartridge vaping product with 16mg/mL nicotine e-liquid, smoking a cigarette and using a heated tobacco product (HTP) (74). Statistically significant differences between all 3 conditions were also reported, with HTP use increasing 8-isoprostane levels least and smoking increasing 8-isoprostane levels most. Two other studies found no statistically significant differences in 8-isoprostane levels between different study conditions. Mastrangeli and others (73) also identified that having a longer smoking history was the strongest predictor of higher 8-isoprostane levels among participants. Benowitz and others (75) argued that the 48-hour abstinence condition could have been too short for urinary 8-isoprostane levels to change (75). Finally, Cobb and others reported that only 28% of 8-isoprostane samples in exhaled breath condensate were above the lowest limit of detection (3.0 picograms per millilitre (pg/mL)), and no statistical comparisons could be made (30).

Four cross-sectional studies, likely to pick up longer-term changes, compared 8-isoprostane levels between vapers, smokers and non-users (67, 76-78). Two studies reported higher 8-isoprostane levels in smokers compared with vapers' groups (67, 77). Sakamaki-Ching and others did not find statistically significant differences in urinary 8-isoprostane levels between vapers and smokers but reported that 8-isoprostane levels were significantly higher among vapers and smokers compared with levels in non-users (76). Singh and others also reported 22% higher 8-isoprostane levels in exhaled breath condensate of vapers than non-users, but this difference was not statistically significant (78). In addition, Sakamaki-Ching and others reported that 8-isoprostane levels were statistically significantly elevated among participants older than 40 years of age and

among women compared with men, suggesting that age and gender might be associated with higher sensitivity to oxidative stress (76).

The one study that included smokers with asthma was a longitudinal study which reported on 8-isoprostane level changes in exhaled breath condensate of healthy smokers and smokers with asthma after they were exposed to 10 puffs of a cartridge vaping product with 10mg/mL to 15mg/mL nicotine e-liquid (79). The study authors reported a statistically significant increase in 8-isoprostane after exposure to vaping product use in smokers with asthma and a non-significant increase in healthy smokers, although variability within these groups remained high and no statistically significant difference in changes between the 2 groups was found (79).

White blood cell (WBC) count

Eight studies were identified in our systematic review that assessed WBC count, one of which included people with an asthma diagnosis.

A RCT (80) reported on changes in various WBC count in non-users (aged between 21 and 30 years who had never smoked or smoked fewer than 100 cigarettes in their lifetime and had not vaped within the past year) exposed either to vaping (n=14) a non-nicotine, non-flavoured 50% PG/VG e-liquid for 4 weeks of daily use, or non-use. No statistically significant changes were reported in total WBC count and in count of macrophages, lymphocytes, neutrophils and eosinophils within vaping product users and non-users' groups at 4-week follow-up. However, changes in urinary propylene glycol (as a marker of vaping product use and inhalation) were significantly correlated with changes in blood cell concentrations, macrophage count (borderline significance) and lymphocyte count, although the absolute magnitude of changes was small.

A longitudinal study of 24 months found no statistically significant changes in WBC count at all follow-up points compared with baseline after smokers switched to ad libitum use of a cartridge vaping product with 16mg/mL nicotine, among the full analytical sample, those that completed the study, and those that were largely compliant (self-reported abstinent on 80% or more of study days) (60).

Six cross-sectional studies (also likely to pick up longer-term changes) reported on all or some types of WBC count (77, 81-85). A study with the highest sample size (n=1208), which included only men, found no difference in WBC count between self-reported vapers and smokers but reported statistically significantly lower WBC count in non-users (81). Oliveri and others (77) found a 9% lower WBC count among vaping product users than smokers, but this difference was not statistically significant. Sahota and others (82) reported no differences in WBC count between vapers, smokers and non-users. Song and others (83) in their bronchoscopy study reported that the macrophage count was significantly higher in smokers compared with vapers and no significant differences between vapers and non-users; for lymphocytes, neutrophils and eosinophils there were

no significant differences between the vapers and non-users, statistically significant higher levels of lymphocytes observed in non-users compared with smokers, and higher levels of neutrophils in smokers compared with never smokers, and no statistically significant difference for eosinophils. Tsai and others (84) reported no statistically significant differences in the macrophage count between vapers and smokers (p=0.06) and non-users; for lymphocytes and neutrophils, there was no significant difference between all groups.

In relation to our second research question, the cross-sectional study by Aboelnaga and others (85) studied participants diagnosed with asthma but without a respiratory infection or asthma exacerbation within the last 2 months. The authors reported a statistically significant difference between groups for the eosinophil count with the non-users' group greater than the vapers' and smokers' groups and no difference between the vapers' and smokers' groups.

C-reactive protein (CRP)

Seven studies were identified in our systematic review that assessed CRP changes.

One RCT reported on high-sensitivity CRP changes in smokers of at least 15 cigarettes per day who for 4 weeks switched to using a cartridge vaping product with 16mg/mL nicotine e-liquid, the same vaping product with 0mg/mL nicotine e-liquid, or continued to smoke (86). The RCT did not find significant changes in CRP levels within or between study arms at 4 weeks follow-up (86).

Chatterjee and others (87) explored changes in blood serum CRP levels after healthy non-smokers were exposed to 16 puffs of a disposable vaping product with 70%/30% PG/VG ratio, tobacco-flavoured and 0mg/mL nicotine e-liquid. The study reported statistically significant increases in CRP levels after acute exposure to vaping product use without nicotine. The authors also noted considerable variation in inflammation markers at baseline (due to age, sex, weight, lipid levels, blood pressure, fitness, and antioxidant status) and concluded that the acute phase CRP increase by 20% to 25% after vaping product use was comparable to inflammatory disorders (87).

Five cross-sectional studies (that are likely to pick up longer-term changes) reported on vaping associations with CRP levels (67, 78, 81, 88, 89), and as discussed in chapter 8, we pooled data from 2 studies (67, 88) comparing vapers and smokers' CRP levels for meta-analysis (figure 4, chapter 8). Pooled data showed statistically significantly lower average blood CRP levels in vapers compared with smokers (LMD: -0.29, 95% CI: -0.43, -0.15; 628 participants); the geometric mean CRP levels were approximately 25% lower among vapers than among smokers (GMR: 0.75, 95% CI: 0.65, 0.86) and heterogeneity between the 2 studies was low (I^2: 0%). Two other studies reported similar blood (81) and salivary CRP (89) levels between vapers and smokers, with large variance within study groups. Regarding CRP differences between vaping products users and non-

users, again as described in chapter 8, we pooled data from 2 studies (67, 88). No statistically significant difference was found between average blood CRP levels in vapers and non-users (LMD: -0.17, 95% CI: -0.51, 0.17; 535 participants). Heterogeneity between the 2 studies was substantial (I^2: 79%). The other 3 cross-sectional studies also did not find statistically significant differences in CRP levels between vapers and non-users (78, 81, 89).

Interleukins (IL-6 and IL-8) and tumour necrosis factor alpha (TNF-α)

Twelve studies were identified in our systematic review that assessed changes in IL-6, IL-8 and TNF-α levels, one of which compared healthy smokers with smokers with asthma.

The one RCT (described above for WBC count) which assessed IL-6, IL-8 and TNF-α levels, randomised non-users (aged between 21 and 30 years who had never smoked or smoked fewer than 100 cigarettes in their lifetime and had not vaped within the past year) either to vaping (n=14) a non-nicotine, non-flavoured 50% PG/VG e-liquid for 4 weeks of daily use, or non-use (n=13). The authors reported that the inflammation markers IL-6, IL-8 and TNF-α did not change statistically significantly and did not differ from the non-users' group at the 4-week follow-up (80). However, changes in urinary propylene glycol (as a marker of vaping product use and inhalation) were significantly correlated with changes in IL-8 and TNF-α only, although the absolute magnitude of changes was small.

One cross-over study reported vaping associations with IL-6 and IL-8 (75). Benowitz and others (75) reported that, compared with 48-hour abstinence condition, participants' blood plasma IL-6 and IL-8 levels were statistically significantly higher after ad libitum vaping or smoking conditions, with no difference between the latter 2 conditions. The authors also reported no differences in IL-8 levels between users of different types of vaping product and found statistically significantly higher blood IL-6 levels in participants who used a modular versus cartridge type vaping product.

Nine cross-sectional studies (that are likely to pick up longer-term changes) reported on IL-6 and/or IL-8 and/or TNF-α levels associated with vaping (67, 78, 83, 89-94) and the findings were mixed. The methods of assessing IL-6 and IL-8 levels and the findings differed across the included studies, and TNF-α was assessed in different biosamples. The study with the largest sample size (n=1857) reported on blood plasma IL-6 levels among women between 18 and 49 years old who were self-reported vapers, smokers or non-users of tobacco and nicotine products (67); the study found no statistically significant differences in IL-6 levels between the 3 groups. One other study also reported no statistically significant differences in salivary IL-6 and IL-8 levels and TNF-α levels, between college students (age range 18 to 25) who were self-reported vapers in the past 30 days and those who did not use vaping products (90). A further study reported no statistically significant differences in salivary IL-6 and IL-8 levels between vapers, 'dual users', smokers and non-users (89), but reported statistically significantly lower levels of TNF-α in the non-user group compared with the other 3 groups. One study reported higher

blood plasma IL-6 and IL-8 levels among exclusive vapers compared with non-users (78). A further study that used BAL found statistically significantly higher IL-6 levels in self-reported vapers compared with non-users; no statistically significant difference in TNF-α levels were observed between vapers, smokers and non-users, whereas IL-8 levels were statistically significantly higher in smokers compared with non-users with no differences between vapers and smokers or vapers and non-users (83). One study (91) reported statistically significantly higher IL-6 levels and TNF-α levels in gingival crevicular fluid of smokers compared with vapers, and significantly higher TNF-α levels in smokers than non-users. Three studies included participants with at least one dental implant or a diagnosis of periodontitis (92-94). One study reported statistically significantly higher IL-6 and TNF-α levels in the peri-implant sulcular fluid of ‘dual users’ compared with non-users (92). Another study reported statistically significantly higher IL-8 levels in gingival crevicular fluid of vapers than smokers, but lower than in non-users' group, whereas TNF-α levels were significantly higher in smokers than vapers (93). The final study which only assessed TNF-α levels reported no statistically significant differences between users of vaping products and non-users (94).

The study (79) which compared smokers with asthma with smokers without asthma was an acute interventional study which examined Il-6, IL-8 and TNF-α levels, 15- and 30-minutes following exposure to 10 puffs of a cartridge vaping product with medium nicotine content. No changes in these biomarkers were reported between the 2 groups after exposure, and only TNF-α levels increased statistically significantly after vaping product use in smokers with asthma, but not in smokers without asthma. Nevertheless, the study authors concluded that vaping product use altered airway inflammation in smokers with asthma more than in healthy smokers based on changes in a few other inflammation markers.

Fibrinogen

In a cross-over study, Moheimani and others (95) explored changes in blood fibrinogen levels of healthy non-users who were exposed to 60 puffs of a tobacco or strawberry flavoured cartridge vaping product with 12mg/mL or 0mg/mL nicotine e-liquid or to sham vaping (without e-liquid) of the same vaping product device. The study authors reported no statistically significant differences in fibrinogen levels within and between study conditions after acute exposure sessions (95).

Three cross-sectional studies compared blood fibrinogen levels between vapers, smokers and non-users (67, 78, 88) and all 3 found no statistically significant differences compared with the comparison groups.

Summary of cross-cutting biomarkers of potential harm

There were mixed results for 8-isoprostane levels (a marker of oxidative stress) and a few studies suggested that other factors such as participants’ longer past smoking history,

older age and female gender might be associated with elevated 8-isoprostane levels, meaning that drawing conclusions from these studies for vaping is complex. In the one longitudinal study with smokers with asthma and healthy smokers, high variability in 8-isoprostane levels was reported within these 2 groups and no statistically significant differences between the 2 groups in changes in 8-isoprostane levels before and after exposure to vaping product use were reported. In general, evidence from the included studies did not suggest strong associations between vaping and 8-isoprostane levels.

In relation to inflammation, several different biomarkers related to respiratory disease (WBC count, CRP, IL-6, IL-8 and TNF-α levels) were studied and again findings were mixed. Considering WBC count, evidence from one RCT suggested that levels of WBC do not change after non-users vaped PG/VG e-liquid without nicotine, and a longitudinal study did not find changes in WBC count 24 months after smokers switched to nicotine vaping product use, although some participants continued smoking. Evidence from the 6 cross-sectional studies was mixed although most reported no statistically significant differences between the different user groups. In the one cross-sectional bronchoscopy study however, the macrophage count, lymphocytes, neutrophils and eosinophils were all statistically significantly higher in smokers compared with vapers and no significant difference between vapers and non-users. In the one cross-sectional study which included participants with an asthma diagnosis there was no difference in eosinophil count between the vapers' and smokers' groups, whereas both were statistically significantly lower than the non-users' groups.

In relation to CRP levels, one RCT found no statistically significant differences in high-sensitivity CRP levels within or between groups 4 weeks after smokers switched to vaping product use with nicotine, vaping product use without nicotine or continued smoking. The one acute interventional study among non-smokers reported a statistically significant increase in CRP levels after exposure to a vaping product without nicotine. The cross-sectional studies, likely to pick up longer-term effects, observed mixed results. However, 2 cross-sectional studies eligible for meta-analysis, showed lower blood CRP levels among vapers than smokers and similar levels between vapers and non-users.

Studies assessing changes in Il-6 and/or IL-8 and/or TNF-α levels, after exposure to vaping products also reported mixed findings. The RCT which randomised non-users to 4 weeks of daily PG/VG vaping (without nicotine and flavours) or non-use reported no statistically significant change in either group and no differences from the non-users at the 4-week follow up for Il-6, IL-8, and TNF-α levels. This study also assessed gene expression in lung epithelial levels and similarly reported no within and between group changes (described in chapter 9 on cancer alongside the cross-sectional bronchoscopy study by the same authors also examining DNA methylation and gene expression). The 9 cross-sectional studies that assessed at least one of these 3 biomarkers had no consistent findings. An acute longitudinal study that assessed IL-6, IL-8 and TNF-α levels, only found a statistically significant increase in TNF-alpha levels among smokers with asthma and not

among smokers without asthma. There were no statistically significant changes in fibrinogen in included studies.

Overall, findings for the biomarkers of potential harm that cut across several diseases including respiratory diseases, were mixed.

Biomarkers of potential harm with specific relevance to respiratory disease

Study characteristics

Our literature search identified 25 unique studies which assessed biomarkers of potential harm associated specifically with respiratory disease (36, 38, 42, 46, 51, 52, 60, 61, 78, 79, 85, 96-109). All 25 studies are summarised in table 1. One study was a randomised controlled trial (RCT) (96), 6 cross-over trials (36, 42, 103-106), including one with participants diagnosed with asthma (106), 13 non-randomised longitudinal studies (38, 46, 51, 52, 60, 61, 79, 97-102), including 2 non-randomised longitudinal studies with different follow-up lengths of a cohort of people with COPD (97, 98), 2 longitudinal studies with people with asthma diagnosis (79, 99), one longitudinal study with people with mental health diagnosis (61), and 5 cross-sectional studies (78, 85, 107-109), including one with people diagnosed with asthma (85). Many of these studies also reported biomarkers of other diseases discussed in other chapters.

Of the 25 studies, 3 were carried out in the UK (36, 60, 61), 7 in the US (78, 96, 100-102, 108, 109), 5 in Italy (51, 97-99, 103), 2 each in Belgium (42, 104) and Greece (79, 106), one each in Egypt (85), Germany (46), Hungary (52), Poland (38), Saudi Arabia (107) and Sweden (105). Of all included studies, one non-randomised longitudinal study was supported by the tobacco industry (60).

Sample sizes of the included studies ranged from 10 in 2 non-randomised longitudinal studies (99, 102) to 263 in an RCT (96). Participants' mean age ranged from 21 in a non-randomised longitudinal study (101) to 66.9 in a non-randomised longitudinal study with people with COPD (51), and between 0% (36, 42, 52) and 60% (96, 105) of participants were females (table 1).

All 25 studies included some lung function measurements, 8 studies assessed FeNO levels and 4 additional studies included imaging or bronchoscopies.

RCT

In the one included RCT, Veldheer and others (96) recruited 263 participants (mean age 47 years) who had smoked an average of 18.4 cigarettes per day for over one year. They were randomised to receive either 36mg/mL nicotine vaping product, 8mg/mL nicotine vaping product, non-nicotine vaping product or a plastic cigarette-like tube that emitted no

vapour. Those in the vaping arms were given a tank vaping product (3.3 volts, milliampere hour (mAh) battery, 1.5 ohm (Ω) dual coil) with 70%/30% PG/VG. Respiratory biomarkers (FEV1, FVC, FEV1/FVC) were assessed at one- and 3-months follow-ups (table 1).

Cross-over

Across the 6 cross-over studies (36, 42, 103-106), 174 participants were recruited. Three recruited healthy occasional smokers (36, 104, 105), one study recruited both healthy smokers and smokers who had diagnosis of asthma (106), one study recruited people who had never used tobacco products (103) and the other study reported former tobacco smokers who had been using vaping products for one year or longer (42). Two studies assessed nicotine vaping and non-nicotine vaping conditions (42, 105), with Chaumont and others (42) also having a non-use condition. Two studies assessed nicotine vaping product use and non-use (sham vaping) (104, 106), and the other 2 studies assessed nicotine vaping product versus smoking (36, 103), with Coppeta and others (103) exposing non-users to tobacco and vaping products. All assessed FEV1 and nearly all studies, except for Antoniewicz and others (105), also tested FEV1/FVC. Four studies also tested PEF (36, 42, 104, 106), and 2 studies tested FeNO (105, 106) (table 1).

Longitudinal

Across the 13 non-randomised studies (38, 46, 51, 52, 60, 61, 79, 97-102), 822 participants were recruited.

Two studies included people with COPD who smoked and people with COPD who vaped (97, 98). Another study included outpatients with an asthma diagnosis who had recently switched from smoking to vaping (99), and a further study included smokers with an asthma diagnosis as well as healthy smokers (79). One study explored participants who were smoking and had an established clinical mental health diagnosis, including schizophrenia, schizoaffective or bipolar disorder (61).

The remaining studies included general population participants who were vapers, ‘dual users’, smokers or non-users. Five studies (38, 79, 100-102) assessed exposure to vaping after single use (nicotine vaping and/or own brand and/or non-nicotine and/or sham vaping, and/or no intervention) and one of these (100) also included non-users’ exposure to passive vape exposure. Seven studies followed up participants over periods of time: for 7 days, which included current vaping product users who switched to tobacco smoking (52), for 3 months (46), for 24 weeks, which included participants with mental health diagnosis (61), for 6 months, which included outpatients with asthma (99), 24 months (60), 36 months, which included participants with COPD (98), 42 months (51) and 60 months, which also included participants with COPD (97). All studies assessed FEV1, FVC, FEV1/FVC except Walele and others (60) (no FEV1/FVC) and McClelland and others (100) (just FVC). Additionally, 5 studies (38, 46, 51, 79, 99) assessed FeNO; and 6 studies (38, 52, 60, 61, 79, 99) also tested PEF (table 1).

Cross-sectional

Findings on respiratory biomarkers were assessed in 4 cross-sectional studies that included 280 participants and were reported in 5 publications (78, 85, 107-109). Aboelnaga and others (85) included participants diagnosed with asthma but without a respiratory infection or asthma exacerbation within the last 2 months, who were categorised as users of vaping products, people who smoked, or non-users. Meo and others (107) included people who self-reported as using vaping products or as never having tried vaping products or cigarettes. Singh and others (78) included healthy participants without chronic diseases or respiratory infections categorised as people who used vaping products or never users of tobacco products. Finally, both studies by Ghosh and others (108, 109) included self-reported vaping product users, people who smoked and people who reported never smoking, who all underwent bronchoscopies. All 4 studies assessed FEV1 and FVC, with Meo and others (107) and Singh and others (78) also testing FEV1/FVC and PEF. Meo and others (107) also tested FeNO and Ghosh and others (108, 109) also reported on FVC (table 1).

Risk of bias in included studies

RCT

The single included RCT (96) was assessed to have some concerns in overall risk of bias according to the RoB2 risk of bias tool (appendices: table 1). The RCT was assessed to have some concerns due to a lack of information on the randomisation process, missing outcome data and the lack of pre-specified data analysis plan.

Cross-over studies

All 6 included cross-over studies were assessed to have some concerns regarding overall risk of bias (36, 42, 103-106) according to the RoB2 risk of bias tool for cross-over studies (appendices: table 2). Most common concerns were related with a lack of pre-specified data analysis plan.

Longitudinal studies

Among the included non-randomised longitudinal or acute exposure studies, 3 were assessed at low risk of bias (52, 79, 102), 7 at moderate risk of bias (38, 46, 51, 60, 97-99) and 3 at serious risk of bias (61, 100, 101) according to the ROBINS-I risk of bias tool for non-randomised longitudinal studies (methods: table 3). The study by Kizhakke Puliyakote and others (101) was assessed to have serious risk of bias in classification of interventions domain, McClelland and others (100) was assessed to be at serious risk of bias due to confounding by smoking, which was not accounted by analysis methods, and a study by Hickling and others (61) was assessed to be at serious risk of bias due to bias in selection of the reported result.

Cross-sectional studies

The quality of the included cross-sectional studies was assessed using Biocross quality appraisal tool and is reported in the appendices (table 4). The included studies were rated from 5 (85) to 14 (108, 109) out of 20 in terms of their risk of bias. The main limitations were associated with study population representativeness (lack of sampling frame definition, sample size justification or information about response rate) and lack of discussion on limitations arising from the cross-sectional study design.

Study findings

Results are presented by outcome measure, and for each measure we initially address the first research question by summarising findings from the general population studies, and then the second research question by focusing on studies that recruited people with existing respiratory diseases. It should be noted that we did not identify eligible studies for meta-analysis following the algorithm described in the methods chapter (methods: table 6), therefore in this chapter all the studies are narratively synthesized.

Spirometry measures

All 25 studies included some spirometry measures. The one RCT (96) encouraged smoking reduction by providing vaping and cigarette substitute products, and, at 3 months, only 6 people in the combined vaping product group (including those using 0mg/mL, 8mg/mL and 36mg/mL nicotine e-liquids) and one in the substitute group exclusively used the allocated products. The analysis compared changes within the combined vaping product groups and the substitute group between baseline and follow-ups adjusting for socio-demographics, cigarettes smoked per day and time of follow-up, group, time of product usage and days using the study products. There was no statistically significant difference within the vaping product or the substitute group at one- and 3-months follow-up for FEV1 and FEV1/FVC. A statistically significant decrease was reported for FVC within the substitute group at 3, but not at one-month follow-ups, and there were no statistically significant changes within the vaping product group at one- and 3-months follow-up.

All the cross-over studies assessed respiratory effects after acute (single use) exposure to vaping products. In the only cross-over study from the UK, Kerr and others (36) observed no statistically significant changes in FEV1, FVC and FEV1/FVC after at least daily smokers were exposed to acute vaping with tobacco-flavoured 18mg/mL nicotine e-liquid or acute tobacco cigarette smoking conditions. Antoniewicz and others (105) observed a non-statistically significant increase in FEV1 within the vaping 19mg/mL nicotine e-liquid and non-nicotine vaping conditions (neither had any added flavourings) among healthy occasional smokers and no difference between the groups over 120 minutes after exposure. In Chaumont and others (104), there was a statistically significant decline in FEV1 and FEV1/FVC among occasional smokers in the non-nicotine unflavoured vaping condition after exposure compared with baseline; no statistically significant difference in FEV1 was reported between the non-nicotine unflavoured vaping and the sham vaping

conditions after exposure, whereas FEV1/FVC was statistically significantly lower after exposure in the non-nicotine unflavoured vaping product condition compared with the sham vaping condition. There were no statistically significant differences after exposure in PEF compared with baseline in both groups and after exposure between groups. Chaumont (42), by contrast, assessed vapers, who were former smokers, and reported that there were no statistically significant differences in FEV1 levels, FEV1/FVC and PEF when they were exposed to vaping 1.5mg/mL nicotine, non-nicotine vaping (flavouring not reported for either condition) and sham vaping conditions 3 hours after exposure. Coppeta and others (103) found that FEV1 levels and FEV1/FVC were statistically significantly lower after non-users (former smokers were excluded) were exposed to a vaping product with tobacco-flavoured 18mg/mL nicotine condition and when exposed to a tobacco smoking condition at one minute after exposure compared with baseline; FEV1 levels were also statistically significantly lower 15 minutes after exposure in the smoking condition compared with baseline, but not the nicotine vaping product condition (103). The authors did query however whether statistical significance translated into clinical significance.

In the single use exposure studies, Staudt and others (102) reported no statistically significant differences in FEV1, FVC levels and FEV1/FVC within groups of never smokers who were exposed to either a single episode of nicotine vaping (n=7) or a single episode of vaping non-nicotine products (n=3) (flavour not reported). Brozek and others (38) also found no significant difference in FEV1, FVC and FEV1/FVC within groups of people who smoked, people who vaped, people who smoked and vaped, and non-users after they were exposed to 5 minutes ad libitum use of tobacco cigarettes, 12mg/mL nicotine and multi-fruit flavoured vaping products, both vaping and smoking products and simulated vaping respectively, one minute and 30 minutes after exposure. The authors also did not observe any differences in spirometry measures between different participant groups at baseline and concluded that it might be due to the relatively young participants' age (mean age: 22.6), as more pronounced decline in lung function measures is visible among longer-term smokers (38). A longitudinal study by Kizhakke Puliyakote and others (101) observed that at baseline FEV1 and FEV1/FVC were statistically significantly higher among people who vaped daily for more than one year (n=9) compared with non-smokers/non-vapers (n=7), but no significant differences were found in FVC levels. This finding is contrary to expectations, but a significant limitation of this study was that of the 9 vapers, 5 subjects reported infrequent hookah use and 8 reported marijuana use (one reported vaping, 3 reported smoking, and 4 both methods). Again, the participants were relatively young (mean age: 23 years). McClelland and others (100) assessed firsthand and secondhand vaping effects in people who vaped (mint-flavoured 5% nicotine strength) and non-users who were in the same room. No statistically significant changes in FVC were observed within groups between baseline and after exposure to ad libitum use of a nicotine vaping product for 20 minutes, and no statistically significant differences reported between the groups after exposure.

Ruther and others (46) reported no statistically significant changes in FEV1, FVC or FEV1/FVC within and between groups of smokers who for 3 months switched to ad libitum vaping their own-brand vaping product and smokers who stopped smoking; to note, 72% and 36% of participants in vaping and non-use groups reported still smoking, which might have affected spirometry measures at 3 months follow-up.

Walele and others (60) in the only UK longitudinal study sent a sample of smokers (n=206) a study vaping product for ad libitum use (tobacco or menthol flavoured 16mg/mL nicotine e-liquid); 102 were followed up at 2 years. They reported a statistically significant decline at 3, 6, 12, 18 and 24 months in FEV1 levels and FVC levels (except at 3 months when there was no statistically significant difference reported), and a statistically significant decline at 12 and 24 months in PEF levels, in those followed up compared with baseline for the full sample. Similar declines, although not statistically significant were reported for the study completers (n=102 for baseline and most follow ups) and for those who were regarded as compliant (n=110 baseline declining to 71 at 2-year follow-up who self-reported abstinence from smoking for at least 80% of the study days, and additionally at study visits had expired air carbon monoxide values of 8 or fewer parts per million (ppm)). The authors commented that these were not judged to be clinically significant changes overall and that the compliant group, who had smoked fewer cigarettes than the full analytical sample ‘showed similar or lower declines confirmed the positive effect of smoking reduction, even if accompanied by vaping’ although it is not clear if the changes in spirometry were compared across groups. However, without a control group, it is not possible to differentiate vaping effects from those of aging.

Polosa and others (51) followed up people who had smoked less than 100 cigarettes in their lifetime but vaped for at least 3 months (products of varying nicotine strength and flavours) at baseline (n=9) and age- and sex-matched non-users of nicotine and tobacco products (n=12) for 3.5 years. They reported no statistically significant difference in FEV1, FVC or FEV1/FVC between the 2 groups at 12, 24 and 42 months follow up points. Authors, however, noted that the small sample size (n=21) and relatively young age of participants (~27 years old on average) might preclude from making strong conclusions about the reported similarities in respiratory function between vapers and non-users (51).

Finally, Barna and others (52) asked former heavy smokers who were current users of nicotine vaping products to switch back to smoking 2 to 25 cigarettes per day for 7 days and reported that FEV1 or FVC levels were statistically significantly higher at baseline compared with post smoking; no statistically significant differences were found between baseline and after a week of smoking for FEV1/FVC and PEF.

In the 3 cross-sectional studies, likely to pick up longer-term effects on lung function, Ghosh and others (108, 109) reported no statistically significant differences in FEV1 and FVC levels between vapers (former or never smokers who reported using a vaping product for 1 to 2.5 years), smokers and never smokers. However, there was significant

confounding in the study due to non-users being able to smoke less than 4 cigarettes a week as well as the vaper and smoker categories not being exclusive users. Singh and others (78) also reported no statistically significant differences in FEV1, FVC FEV1/FVC and PEF between people who were exclusive vapers and never users. Meo and others (107) reported statistically significantly lower FEV1 and FEV1/FVC among people who used vaping products daily for longer than 6 months compared with people who had never smoked or vaped, but no statistically significant differences in FVC or PEF levels. To note, all 3 studies recruited relatively young participants (mean ages between 26 and 34 years old), which could have been an important factor comparing spirometry measures between vapers, smokers and non-users.

Of the 4 studies that included participants with a diagnosis of asthma, one was a cross-over trial which reported baseline spirometry measures only (106), one was an acute exposure study (79), one longitudinal (99) and one cross-sectional study (85), and sample sizes were generally small. In a cross-sectional analysis at baseline of a cross-over study, Lappas and others (106) who only assessed spirometry measures at screening, showed that smokers with mild asthma diagnosis might have worse spirometry measures than smokers without asthma. Study authors reported statistically significantly lower FEV1, FEV1/FVC and PEF levels in smokers with asthma (n=27), compared with smokers without asthma (n=27); no statistically significant differences were observed for FVC between the 2 groups. In a cross-sectional study, Aboelnaga and others (85) assessed 130 participants diagnosed with asthma (but without a respiratory infection or asthma exacerbation within the last 2 months) and reported that FEV1, FVC, FEV1/FVC and PEF levels were statistically significantly higher among non-users than among vapers and smokers, but there were no consistent differences reported between the smoker and vaper groups. Kotoulas and others (79) reported no statistically significant differences within and between groups of smokers with an asthma diagnosis (n=25) and smokers without an asthma diagnosis (n=25) in FEV1, and FVC levels 15 minutes after they used a ‘medium’ nicotine strength vaping product (flavour not reported). However, for PEF and FEV1/FVC, statistically significant decreases were observed within the smokers with asthma after exposure, but no significant difference within the smokers without asthma group or between the 2 groups after exposure. Solinas and others (99) followed up outpatients (n=10) with asthma who recently switched from smoking to vaping and reported no statistically significant differences between baseline and 3 and 6 months follow ups in FEV1, FVC, FEV1/FVC and PEF.

In the 2 longitudinal articles taken from the same cohort of COPD patients in Italy (97, 98), it should be noted that there was considerable use of both vaping and tobacco cigarette products in the vaping group, although findings were reported separately for each group but the small sample sizes preclude conclusions being made. Polosa and others reported no statistically significant differences in FEV1 and FVC levels within people who vaped (n=20) and people who smoked (n=19) at 12, 24 and 36 month follow ups in the first article (98); for FEV1/FVC, there was a statistically significant decrease compared with baseline

within the smokers' group at 12, and 24 months but no difference at 36 months. However, when the same group of patients were followed up at 60 months (97), a statistically significant decrease in FEV1 levels was reported within the group who used vaping products at 12 months, and a statistically significant increase at 48 and 60 months, all compared with baseline; there were no significant changes in the smoking group at any follow-ups. For FVC there was a statistically significant increase at 12-, 48- and 60-month follow-ups within the group who used vaping products, but as for FEV1, there were no significant differences within the group who smoked tobacco products at all follow ups compared with baseline. For FEV1/FVC, there were no significant differences within the vaping product group at all follow ups and a statistically significant decrease was reported for the smoking group at 12 and 24 months compared with baseline. There was a significant difference between the 2 groups from baseline at the final follow up with higher FEV1 and FVC levels and FEV1/FVC reported among people who vaped than among people who smoked. Finally, among the people who vaped, an analysis of people who were exclusive vapers indicated significant increases at 48 and 60 months in FEV1 and FVC levels but not FEV1/FVC, compared to baseline, but no significant differences were observed among the people who smoked and vaped at these follow-up points (97).

One longitudinal study from the UK (61) explored changes in PEF across smokers with a mental health diagnosis after they were encouraged to switch to vaping product use. The study reported no statistically significant changes in PEF 6, 10 and 24 weeks after participants ad libitum used vaping products; at all follow-ups, most of the participants were also smoking.

Fractional exhaled nitric oxide (FeNO)

Eight of the included studies assessed FeNO. In the only cross-over study that assessed FeNO, Antoniewicz and others (105) reported a statistically significant increase in FeNO within the vaping unflavoured 19mg/mL nicotine e-liquid and non-nicotine vaping conditions among healthy occasional smokers, and no difference between the groups over 120 minutes after exposure. Among the longitudinal studies, Brozek and others (38) found statistically significantly lower levels of FeNO within groups of people who smoked, people who vaped, people who smoked and vaped, and non-users after they were exposed to 5 minutes ad libitum use of tobacco cigarettes, multi fruit flavoured 12mg/mL nicotine vaping products, both vaping and smoking products and simulated vaping respectively one minute after exposure compared to baseline. No statistically significant differences within the smoker and dual user groups were reported 30 minutes after exposure compared with baseline, but statistically significantly higher levels of FeNO were reported within the vaping product group 30 minutes after exposure compared with baseline. Ruther and others (46) reported no statistically significant changes in FeNO between smokers who for 3 months switched to ad libitum vaping and smokers who stopped smoking at 3 months follow-up, but again noting the confounding with continued smoking in both groups. As previously reported, Polosa and others (51) followed up people who had smoked less than 100 cigarettes in their lifetime but vaped for at least 3 months at baseline (n=9) and age-

and sex-matched non-users of nicotine and tobacco products (n=12) for up to 3.5 years. They reported no significant differences in FeNO, although the small sample size and relatively young age of the participants that could have confounded study results. Of the 3 cross-sectional studies, only Meo and others (107) compared FeNO levels between people who used vaping products daily for longer than 6 months and people who had never smoked or vaped and reported no statistically significant differences between the groups.

Three of the 4 studies that included participants with a diagnosis of asthma assessed FeNO. A cross-over study by Lappas and others (106) reported no statistically significant differences at baseline between smokers with a mild asthma disgnosis and those without asthma. Statistically significant decreases were reported in smokers with and without asthma, in FeNO levels immediately following 5 minutes of tobacco flavoured 11.8mg/mL nicotine vaping and sham vaping conditions. There was a statistically significant decrease within the asthma group at 15 minutes follow-up compared with baseline, whereas no statistically significant difference within the smokers without asthma group. There were no statistically significant differences in FeNO levels at 30 minutes compared with baseline for both groups and no statistically significant differences reported in either group for the sham vaping condition. However, Kotoulas and others (79) reported a statistically significant increase in FeNO levels 30 minutes after they had used a ‘medium’ nicotine content vaping product (flavour not reported) within people who smoked with an asthma diagnosis (n=25) and a statistically significant decrease among people who smoked without asthma (n=25) and a statistically significant difference between the 2 groups after exposure. Solinas and others (99) followed up outpatients with asthma (n=10) who recently switched from smoking to using vaping products and reported no statistically significant differences between baseline and 3 and 6 months follow ups in FeNO levels. The cohort study of patients with COPD did not assess FeNO measures (97, 98).

Impulse oscillometer (IOS) measurements

Antoniewicz and others (105) in their cross-over study among healthy occasional smokers reported that respiratory system flow resistance at 11Hz, 13Hz, 17Hz and 19Hz (R11, R13, R17, R19) statistically significantly increased 30 minutes after exposure within the nicotine (19mg/mL) vaping condition but not the non-nicotine vaping condition (all $p<0.01$) and that there was a statistically significant difference between groups (all $p<0.01$). Resonance frequency (fres) decreased at 6 hours follow-up within the non-nicotine vaping condition, but not the nicotine vaping condition ($p<0.05$). There were no statistically significant differences for the other IOS measures within and between groups.

In their study with smokers with and without asthma, Lappas and others (106) reported no statistically significant differences in impulse oscillometer (IOS) impedance, resistance, and reactance measured before and 0, 15 and 30 minutes following a 5-minute sham vaping condition. Statistically significant increases were reported immediately following 5 minutes of tobacco flavour 11.8mg/mL nicotine vaping, in respiratory system total

impedance at 5Hz (Z5), respiratory system resistance at 5Hz (R5), respiratory system resistance at 10Hz (R10), respiratory system resistance at 20Hz (R20), resonant frequency (fres) and reactance area, (AZ), while respiratory system resistance at 20Hz (X20) decreased, for both groups (smokers with and without asthma); X5 was statistically significantly higher in smokers with asthma only after exposure. All parameters gradually returned to baseline at 15-minute follow-up. Statistically significant higher baseline values and a more prominent effect immediately after the nicotine vaping experimental condition were reported for the smoker group with mild asthma compared with the smokers without asthma for Z5, R5 and R10. The authors concluded that a single session of vaping had respiratory mechanical and inflammatory effects, which were more prominent in smokers with asthma.

Kotoulas and others (79) also took IOS measurements before and after exposure to a ‘medium’ nicotine strength vaping product and there was some consistency with the findings reported by Lappas and others (106). Kotoulas and others (79) reported a statistically significant increase within smokers with asthma for Z5 but not within the smokers without asthma group; R5, R10, R20 statistically significantly increased within both groups.

Bronchoscopy and imaging studies

The RCT (80) referred to in the section on Biomarkers of Potential Harm cutting across several diseases above in relation to inflammation changes also assessed gene expression (microRNA, and mRNA from lung epithelial cells) as did the cross-sectional bronchoscopy study by Song and others (83); both these studies are discussed in chapter 9 on cancer. Staudt and others (102) reported above also examined genome-wide gene expression profiles, assessed by mRNA-sequencing of alveolar macrophages collected by bronchoalveolar lavage, among never smokers exposed to a single episode of nicotine or non-nicotine vaping. These findings are again described in chapter 9 on cancer.

In addition to these bronchoscopy studies, 5 articles on 4 studies were identified here which utilised imaging or bronchoscopies to assess different biomarkers of potential harm. Ghosh and others (2018) carried out bronchoscopies on healthy non-smokers, cigarette smokers and vapers and determined protease levels in BAL as well as analysing nicotine concentration in induced sputum and BAL (109). They also carried out some in vitro assessments, also reported later in this chapter. They found that the proteases neutrophil elastase, matrix metalloproteases (MMP)-2 and MMP–9 and protein levels were equally elevated in both vapers’ and smokers’ BAL relative to non-smokers. Antiproteases (specifically alpha-1 antitrypsin and secretary leukocyte protease inhibitor and tissue inhibitors of MMP1 and MMP2) however were not different. After vaping, measurable levels of nicotine were detectable in the sputum and BAL. From the same study, Ghosh (2019) reported that approximately 300 and approximately 200 proteins were significantly altered in smokers’ and vapers’ bronchial epithelia, respectively, and that only 78 proteins were commonly altered in both groups and 113 uniquely altered in vapers (108). The

authors noted that protein groups associated with membranes especially were altered in vapers. Through parallel in vitro research, the authors concluded that the changes may be part by mediated by PG/VG. However, this study was limited as described earlier, in that the non-smokers could smoke up to 4 cigarettes per week, and that the smoker and vaper groups were not exclusive users.

In a study by Kizhakke Puliyakote and others (101), imaging was carried out for both the non-smoker/non-vaper group (n=7) and vaper group (n=9) at baseline (subjects had abstained from vaping for at least 6 hours before the study). Then 8 of 9 from the vaper group vaped a 50mg/mL nicotine salts disposable vaping product (Puff Bar) ad lib with the ninth a 6mg/mL e-liquid. The vapers were imaged again immediately after vaping. The non-user group was only imaged at baseline as they were given no intervention. Mean alveolar ventilation, ventilation heterogeneity and perfusion were similar between the non-smoker/non-vaper group and the vaper group at baseline; mean perfusion heterogeneity and ventilation-perfusion heterogeneity were significantly higher in vapers at baseline compared with the non-smoker/non-vaper group. After vaping, mean alveolar ventilation and perfusion heterogeneity were significantly decreased in vapers, whereas ventilation heterogeneity, mean perfusion and ventilation-perfusion heterogeneity significantly increased. The authors reported that ventilation-perfusion heterogeneity in the vaper group was at the upper limit of normal reported for healthy subjects. However, as reported above, a significant limitation of this study was that of the 9 vapers, 5 subjects reported infrequent hookah use and 8 reported marijuana use (one reported vaping, 3 reported smoking, and 4 both methods) which precludes any conclusions being drawn.

Polosa and others (51) carried out high-resolution computed tomography (HRCT) of the lungs of 8 of the 9 vapers at the last follow-up (3.5 years) to assess risk of early signs of lung damage. Visual assessment showed no pathological findings and no CT features compatible with early signs of COPD, lipoid pneumonia or popcorn lung disease.

In a cross-over study, Barna and others (52) examined lung alveolocapillary membrane using dynamic ventilation scintigraphy among vapers. Clearance rates were significantly faster after switching back to one week of cigarette smoking (and differed significantly across the right and left lung). The authors indicated that the increase in clearance rates represented damage to alveolocapillary membrane function from the smoking.

COPD and asthma severity scales

In the 2 longitudinal studies of the same cohort of COPD patients led by Polosa and others (98) (97), assessments were also made of COPD exacerbations, GOLD COPD staging, CAT scores and a 6-minute walk test (6MWD). While there were small sample sizes, in general improvements were reported for these measures for vapers, but not smokers, at some or all of the follow-ups. While some of the vapers also smoked, some data were presented separately for exclusive and ‘dual users’ but the sample sizes were too small to draw any conclusions.

Aboelnaga and others (85) assessed the Asthma Control Test in their cross-sectional study of asthma sufferers, finding statistically significant higher (improved) scores for non-users compared with vapers and smokers, and that vapers had statistically significantly higher scores than smokers.

Summary of specific biomarkers of respiratory disease

All the studies assessed spirometry measures but the different designs, groups and duration of exposure limits any conclusions that can be drawn. The one RCT included in our systematic review in this section was a smoking reduction trial and only 6 (out of 191) people across the 3 vaping product groups achieved abstinence from smoking at 3 months, and so continued smoking will have confounded the results. There were no statistically significant changes in the lung function measures within the vaping product groups combined at one- and 3-months follow-up. The cross-over studies assessed acute exposure to vaping products only and reported mixed findings, although most observed no significant differences. For example, the one UK cross-over trial found no significant differences from acute exposure to nicotine vaping or tobacco smoking conditions among smokers. Longitudinal studies assessing acute exposure to vaping again largely reported no statistically significant differences in lung function measures. Studies assessing longer-term exposures also largely reported no statistically significant changes in spirometry measures. These included a study of vapers who had smoked less than 100 cigarettes in their lifetime and were followed up for 3.5 years and compared with age- and sex-matched non-users – the authors observed no differences between the 2 groups. The only UK longitudinal study reported statistically significant declines in 3 spirometry measures compared with baseline at various follow ups for up to 2 years among the total analytical sample of smokers who were sent study products and those followed up; similar declines although not statistically significant were observed among those who had largely switched to vaping. There was no control group for comparison limiting the conclusions that can be drawn from this study. One small study of vapers who were asked to switch back to smoking for 7 days reported statistically significant declines for 2 lung function measures and no changes for a further 2 measures. Cross-sectional results were mixed. Finally, only one small study at serious risk of bias assessed second-hand exposure, so no conclusions can be drawn about effects on spirometry measures.

It should be noted that most of these studies had small sample sizes and participants who were generally relatively young, some with average age under 30.

Of the 4 studies that included participants with a diagnosis of asthma, sample sizes were again generally very small and the findings were inconclusive. One cross-sectional analysis in a longitudinal study which only assessed lung function measures at baseline, suggested that people who smoked with mild asthma diagnosis had worse spirometry measures than healthy smokers. Another cross-sectional study that only included people with asthma reported higher (improved) levels in 4 lung function tests among non-users than people who vaped or smoked, with no consistent differences among smokers and

vapers. After acute exposure to vaping there was no statistically significant differences in changes in lung function among smokers with and without asthma. A longitudinal study following up 10 outpatients with asthma who recently switched from smoking to vaping found no significant differences for up to 6 months of follow-up in 4 lung function measures.

In the 2 longitudinal articles taken from the same cohort of COPD patients in Italy (97, 98), a statistically significant increase in FEV1 and FVC levels was reported within the group who used vaping products at the longest follow-up point (60 months) compared with baseline and no significant differences within the group who smoked tobacco products. Whereas there was no significant difference for FEV1/FVC, within the vaping product group at all follow ups, for all 3 lung function measures, there was a significant difference between the 2 groups from baseline at the final follow up with higher FEV1 and FVC levels and FEV1/FVC reported among people who vaped than among people who smoked. Differences seem largely due to changes in the exclusive vaping group compared with the dual use group. Again, only small numbers of participants were involved and the authors suggested larger studies were needed to confirm these findings.

In the one longitudinal study with smokers with a mental health diagnosis observed no changes in PEF up to 6 months after ad libitum vaping although most of the participants also smoked.

Overall, where statistically significant changes were observed in spirometry measures, some study authors queried their clinical significance. Studies should compare findings with standard parameters used to judge clinical significance.

Eight studies assessed FeNO and again involved different designs, groups, exposure duration limiting conclusions. Findings were mixed, but most reported no significant differences across different user groups. In 2 longitudinal studies of single use vaping exposure that assessed smokers with and without asthma, both studies reported some statistically significant changes in FeNO in the 2 groups up to 30 minutes after exposure, but at 30 minutes only one study reported statistically significant differences in changes between the groups. In the study following up 10 outpatients with asthma (99) who had recently switched from smoking to using vaping products, no statistically significant differences between baseline and 3 and 6 months follow ups were reported in FeNO levels. Again, it is unclear whether statistically significant changes translate to clinical significance.

The one study that assessed impulse oscillometry measures among healthy occasional smokers reported some differences between the nicotine and non-nicotine vaping condition suggesting there may be an acute effect of nicotine on some lung function attributes. Two studies assessing acute vaping exposure among smokers with and without asthma both reported that some measures changed among smokers with asthma but not for those among smokers without asthma while other measures changed across both

groups. Impulse oscillometry is an emerging diagnostic test, and more research is needed to assess the clinical significance of the findings.

The 5 imaging and bronchoscopy articles reported here and the 2 from the earlier section on biomarkers of potential harm cutting across several diseases and discussed in chapter 9 on cancer, also had mixed and inconsistent results and were also carried out with different designs, user groups and exposures. Further research is needed in this area.

Finally, 2 studies with adequate sample sizes assessed changes in COPD and asthma severity using validated scales. In the COPD study, in general improvements were reported for measures among vapers but not among smokers at some or all follow-ups. The cross-sectional study that assessed asthma severity reported the highest scores for non-users, followed by vapers and then smokers. These 2 studies suggest that vaping may be less risky to people with these conditions compared with smoking but further longitudinal research is needed in this area.

Table 1. Summary of studies exploring vaping products (VP) use associations with biomarkers of potential harm to respiratory health arranged by study design

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
RCT					
Veldheer et al., 2019, US (96)	3 months (S-M)	n = 263 Smokers of 18.4 CPD for >1 years. Mean age 47, 60% females.	3 months ad libitum use of: Vaping (n=191): a tank VP (3.3 volts, 1000 mAh battery, 1.5 Ω dual coil) with 70%/30% PG/VG and 36, 8 or 0 mg/mL nicotine strength e-liquid. Other (n=72): a plastic cigarette-like tube that emits no vapour (cig-sub).	FUs at 1 & 3 months. This was a smoking reduction trial. At 3 month FU: 69.2% of all participants dually used TC and assigned product, 26.6% exclusively smoked and 2.7% (n=6 VP & n=1 the cig-sub) exclusively used the assigned product. 1.1% (n=3) stopped smoking and using the assigned product. Analysis adjusted for age, race, gender, education, CPD, follow up visit (1 month or 3 months), group (e-cig or cig-sub), e-cig or cig-sub times used, days used the study product. FEV1: NS diff. within VP or cig-sub groups at month 1 & 3 FUs. FVC: Stat. sig. decrease within cig-sub group at month 3 FU (p=0.02). NS diff. within VP group at month 1 & 3 FUs and within cig-sub group at	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				month 1 FU. FEV1/FVC: NS diff. within VP or cig-sub groups at month 1 & 3 FUs.	
Cross-over					
Antoniewicz et al., 2019, Sweden (105)	Single use (A)	n = 15 Smokers: healthy occasional smokers of ≤10 TC per month. Mean (SD) age: 26 (3), 60% females.	Cross-over conditions separated by 1 week. Vaping, nicotine (n=15): 30 3-second puffs across 30 minutes of modular VP (32 W, 0.2Ω, dual coil atomizer), 49.5%/44.4%/5% PG/VG/Ethanol, 19 mg/mL nicotine, without any added flavourings. Vaping, non-nicotine (n=15): same puffing regime on the same VP with 0 mg/mL nicotine.	FEV1: NS increase within VP and nnVP groups over 120 minutes after exposure (p=0.096). NS diff between groups (p=0.788) FeNO: stat. sig increases within VP and nnVP groups over 120 minutes (p=0.022). NS diff. between groups (p=0.067) IOS measures are reported in the narrative.	Some concerns
Chaumont et al., 2019, Belgium (104)	Single use (A)	n = 25 Smokers: healthy occasional smokers of <20 TC per week. Mean (SEM) age: 23 (0.4), 28% females,	Cross-over conditions separated by 1 week. Vaping (n=25): 25 puffs with 30 s inter-puff interval and 4 s inhalation	Respiratory outcomes measured for n=9 participants. FEV1: stat. sig. decline within VP group after exposure compared with baseline (p=0.021).	Low risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		mean (SEM) BMI: 23 (0.4) kg/m2.	on a modular type VP (Alien 220 box mod, TFV8 baby beast tank, a dual Kanthal coil (V8 Baby-Q2 Core; 0.4Ω dual coils; Smoke) with 50%/50% PG/VG ratio unflavoured and without nicotine e-liquid vaporised at 60 W, creating sub-ohm vaping exposure. Sham vaping (n=25): the same puffing regime with turned off VP.	NS diff. between groups after exposure (p=0.187). FEV1/FVC: stat. sig. decline within VP group after exposure compared with baseline (p=0.002). Stat. sig. lower level in VP group compared with non-use group after exposure (p=0.014). PEF: NS diff. after exposure compared with baseline in both groups. NS diff. after exposure between groups.	
Chaumont et al., 2020, Belgium (42)	Single use (A)	n = 30 Vapers: former TC smokers, used VP for ≥1 year. Mean (SD) age: 38 (2), 100% males, mean (SD) BMI: 26 (1) kg/m2.	Cross-over conditions separated by at least 7 days: Vapers, nicotine (n=30): 10 puffs of modular VP (Alien 2020 box mod, 60 W, 0.4 Ω, 3000 mAh) with 50/50 PG/VG ratio liquid with 1.5 mg/mL nicotine. Vapers, non-nicotine (n=30): same use of the same modular VP without	FU 3 hours after exposure. FEV1: NS diff. between groups. FEV1/FVC: NS diff. between groups. PEF: NS diff. between groups.	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
			nicotine. Non-users (n=30): sham vaping of the same modular VP.		
Coppeta et al., 2018, Italy (103)	Single use (A)	n = 30 Non-users: never users of tobacco products. Mean (SD) age: 32.6 (2.8), 43.3% females, mean (range) BMI: 23.2 (18-28.7).	Cross-over conditions 'on different days'. Vaping (n=30): 15 puffs over 5 minutes on a VP (EGO P (L)) with Latakia tobacco flavoured, 18 mg/mL nicotine e-liquid. Smoking (n=30): smoke a TC over 5 minutes (consumption equal to 0.6 mg nicotine, 8 mg tar and 9 mg CO).	FUs at 1 & 15 minutes after exposure. FEV1: stat. sig. lower within VP group 1 min (p=0.03) and NS diff. 15 min post exposure (p=0.36) compared with baseline. Stat. sig. lower within smoking group 1 min (p<0.01) and 15 min (p=0.05) post exposure compared with baseline. FEV1/FVC: stat. sig. lower within VP group 1 min (p=0.01) and NS diff. 15 min post exposure (p=0.39) compared with baseline. Stat. sig. lower within smoking group 1 min (p=0.04) and 15 min (p=0.01) post exposure compared with baseline.	Some concerns
Kerr et al., 2019, UK (36)	Single use (A)	n = 20 Smokers: smoking ≥1 TC per day. Mean (SD) age: 31.6 (10.5), all males,	Cross-over conditions separated by >24 hours. Vaping (n=20): 15 puffs on a tank type VP	FEV1: NS diff. after exposure compared with baseline in both groups. FVC: NS diff. after exposure	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		mean (SD) BMI: 25.7 (5).	(1300mAh, 3.3 V battery voltage) with 66%/34% PG/VG ratio, 18 mg/mL nicotine strength and tobacco flavoured vaping liquid. Smoking (n=20): ad lib smoking of a TC.	compared with baseline in both groups. FEV1/FVC: NS diff. after exposure compared with baseline in both groups. PEF: stat. sig. lower within VP (p=0.019) and smoking (p=0.074) groups after exposure compared with baseline.	
Longitudinal					
Barna et al., 2019, Hungary (52)	7 days (A)	n=24 Vapers: current VP users with ≥10 mg/mL nicotine e-liquid; all were former heavy smokers. Age range 20-64, all males.	Smoking (n=24): all participants switched to smoking 20-25 TC per day for 7 days.	Compliance with smoking assessed with exhaled carbon monoxide and carboxyhaemoglobin levels which were significantly higher at FU than BL. FEV1: stat. sig. difference with VP use (baseline) > smoking (7-day FU, p=0.044). FVC: stat. sig. difference with VP use (baseline) > smoking (7-day FU, p=0.025). FEV1/FVC: NS diff between VP use (baseline) and smoking (7-day FU, p=0.197).	Low risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				PEF: NS diff between VP use (baseline) and smoking (7-day FU, p=0.058). Scintigraphy measures are reported in the narrative.	
Brozek et al., 2019, Poland (38)	Single use (A)	n = 120 Self-reported: Smokers (n=30): mean (SD) age: 23.2 (1.6), 50% females. Dual users (n=30): smoking and VP use. Mean (SD) age: 22.3 (2.7), 26.7% females. Vapers (n=30): VP use. Mean (SD) age: 22.2 (2.3), 36.7% females. Non-users (n=30): non-smoking status. Mean (SD) age: 22.9 (1.9), 50% females.	Vaping (n=30): vapers ad lib used their own-brand VP with 12 mg/mL nicotine and multi-fruit flavoured vaping liquid for 5 minutes. Dual use (n=30): dual users ad lib used their own-brand VP with 12mg/mL nicotine and multi-fruit flavoured vaping liquid for 5 minutes. Smoking (n=30): smokers ad lib smoked a TC (0.6 mg nicotine per TC). Non-use (n=30): non-users simulated use of a VP.	FEV1 (n=28 in each group): NS diff. within groups 1 minute and 30 minutes after exposure. FVC (n=28 in each group): NS diff. within groups 1 minute and 30 minutes after exposure. FEV1/FVC (n=28 in each group): NS diff. within groups 1 minute and 30 minutes after exposure. FeNO: stat. sig. lower levels within all groups 1 minute after exposure compared with baseline (p=0.0001). NS diff. within smokers (p=0.2) and dual users (p=0.5) groups 30 minutes after exposure compared with baseline. Stat. sig. higher level within VP group 30 minutes after exposure compared with baseline (p=0.02).	Moderate risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				PEF: stat. sig. lower level within dual users' group 1 minute after exposure (p=0.003). NS diff. within other groups 1 minute and 30 minutes after exposure.	
Kizhakke Puliyakote et al., 2021, US (101)	Single use (A)	n = 16 Vapers (n=9): self-reported VP use for >1 year, vaping daily flavoured disposable e-cigarettes. Mean (SD) age: 23 (5), 42.9% females, mean (SD) BMI: 25 (5). Non-users (self-reported not smoking or vaping, n=7). Mean (SD) age: 21 (2), 33.3% females, mean (SD) BMI: 24 (4).	Vaping (n=9): eight single ad lib use of disposable VP (Puff Bar) with 50 mg/mL nicotine strength nicotine salts; one subject used a 6mg/mL e-liquid. Non-use (n=7): no intervention.	Respiratory Changes after exposure NR. FEV1: stat. sig. diff. between groups at baseline (p=0.05). FVC: NS diff. between groups at baseline (p=0.2). FEV1/FVC: stat. sig. diff. between groups at baseline (p=0.006). Imaging measures are reported in the narrative.	Serious risk of bias
McClelland et al., 2021, US (100)	Single use (A)	n = 149 Self-reported: Vapers (n=76). Non-users (n=73). Mean (SD) age: 22.1	Vapers and non-users were in the same 13.4 m^2 room (firsthand and secondhand exposure). Vapers (n=76): ad lib use of a pod VP (JUUL) with	Covariates: age, gender, present health, recreational drug use, use of cigarettes or alcohol, mental health treatment, presence of a lung, oral or cardiac disease. FVC: NS diff. within groups after	Serious risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		(7.3), 53.7% females.	mint-flavoured and 5% nicotine strength e-liquid for 20 minutes. Non-users (n=73): exposure to the same VP aerosol for 20 minutes.	exposure. NS diff. between groups after exposure.	
Polosa et al., 2017, Italy (51)	42 months (L)	n = 31 Vapers (n=16): had smoked <100 TC in lifetime and were using a VP for ≥3 months. Mean (SD) age: 26.6 (6), 33.3% females. Non-users (n=15): age- and sex-matched non-users of tobacco and nicotine products. Mean (SD) age: 27.8 (5.2), 33.3% females.	42 months ad lib use of: Vaping (n=9): own-brand tank type VP with 0% (3/9), 0.9% (2/9), 1.2% (2/9), 1.6% (1/9) and 1.8% (1/9) nicotine strength vaping liquid with tobacco (7/9), mint (1/9) or fruit (1/9) flavours. Non-use of nicotine products (n=12).	FUs at 12, 24 and 42 months. Compliance The results below are of the 9/16 (56.3%), and 12/15 (80%) in vapers' non-users' group respectively who were not lost to follow-up or become non-compliant with inclusion criteria during the FU period. FEV1: NS diff. between groups (p=0.3) at all FUs. NS diff. between BL and any FU for vaping group. FVC: NS diff. between groups (p=0.6) at all FUs. NS diff. between BL and any FU for vaping group. FEV1/FVC: NS diff. between groups (p=0.09) at all FUs. NS diff. between BL and any FU for vaping group. FeNO: NS diff. between groups (p=0.89) at all FUs. NS diff. between	Moderate risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				BL and any FU for vaping group. Imaging measures are reported in the narrative.	
Ruther et al., 2021, Germany (46)	3 months (S-M)	n = 80 Smokers: smokers for ≥5 years, smoking ≥10 TC per day, wishing to switch to VP or stop smoking. Vaping (n=60): mean (SD) age: 39.1 (12.8), 26.7% females, mean (SD) BMI: 25.3 (30). Non-use (n=20): mean (SD) age: 44.2 (11.7), 50% females, mean (SD) BMI: 23.9 (3.3).	Vaping (n=60): switch from smoking to ad libitum use of own-brand VP. Non-use (n=20): stopping smoking with a controlled smoking cessation program.	FU at 3 months: 40 (67%) out of 60 followed-up in VP group, 14 (70%) out of 20 followed-up in non-use group. Compliance at 3 months: 11 (28%) out of 40 were exclusive vapers in VP group, 9 (64%) out of 14 stopped smoking in Non-use group. FEV1: NS diff. within and between groups at 3-month FU. FVC: NS diff. within and between groups at 3-month FU. FEV1/FVC: NS diff. within and between groups at 3-month FU. FeNO: NS diff. within and between groups at 3-month FU.	Moderate risk of bias
Staudt et al., 2018, US (102)	Single use (A)	n = 10 Never smokers: self-reported, validated by <2ng/mL nicotine and <5ng/mL cotinine in urine.	Vaping, nicotine (n=7): 10 puffs on a cartridge VP (Blu) followed by other 10 puffs after 30 minutes (nicotine content NR).	FU at 2 hours after exposure. FEV1: NS diff. within both groups after exposure compared with baseline.	Low risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Mean (SD) age: 40.2 (9.7), 50% females, 70% Black, 30% Hispanic.	Vaping non-nicotine (n=3): same regime on the same VP without nicotine.	FVC: NS diff. within both groups after exposure compared with baseline. FEV1/FVC: NS diff. within both groups after exposure compared with baseline. Gene expression measurements are reported in the narrative and in Chapter 9 Cancer.	
Walele et al., 2018, UK (60)	24 months (L)	n = 209 Smokers: self-reported smoking of 5-30 TC per day for ≥1 year. Mean (SD) age among those who switched (n=109): 38.7 (10.2), 44.1% females, mean (SD) BMI: 26.2 (4).	24 months ad lib use of: Vaping (n=209): cartridge VP (Puritane) with 1.6% nicotine strength, 67.5%/30% PG/VG vaping liquid with tobacco or menthol flavour.	FUs at 1, 3, 6, 12, 18 and 24 months. Compliance: 102 (48.8%) out of 209 were followed-up at 24 months and were abstinent from smoking cigarettes for ≥80% of the study days. FEV1: stat. sig. decline at 3, 6, 12, 18 & 24 months compared with baseline ($p<0.05$) FVC: stat. sig. decline at 6, 12, 18 & 24 months compared with baseline ($p<0.05$) PEF: stat. sig. decline at 12 & 24 months compared with baseline ($p<0.05$)	Moderate risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				Similar declines, although not statistically significant, were reported within the study completers (n=102 at BL and most FUs) and those compliant.	
Cross-sectional					
Ghosh et al., 2018 & 2019, US (108, 109)		n = 42 Vapers (n=14): mean (SD) age: 26.1 (8.3), 28.6% females, mean (SD) BMI: 29.8 (6.6) kg/m2. Smokers (n=14): mean (SD) age: 29.5 (5.6), 42.9% females, mean (SD)	Vapers (n=14): self-reported former or never smoker, using a VP for 1-2.5 years. Smokers (n=14): self-reported TC use with mean (SD) 7.76 (5.6) pack-years. Non-users (n=14): self-reported never smokers.	FEV1: NS diff. between groups (p>0.05). FVC: NS diff. between groups (p>0.05). Bronchoscopy measures are reported in the narrative.	14/20

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		BMI: 27.8 (6.1) kg/m2. Non-users (n=14): mean (SD) age: 25.8 (7.3), 71.4% females, mean (SD) BMI: 26.2 (5.9) kg/m^2.			
Meo et al., 2018, Saudi Arabia (107)		n = 60 All male participants. Vapers (n=30): mean (SD) age: 27 (6), mean (SD) BMI: 28.5 (7.3). Non-users (n=30): mean (SD) age: 25.9 (7.7), mean (SD) BMI: 28.8 (6).	Vapers (n=30): self-reported daily use of a VP for >6 months. Non-users (n=30); self-reported never smokers or users of a VP.	FVC: NS diff. between groups (p=0.364). FEV1: Stat. sig. higher in non-users compared with VP group (p=0.007). FEV1/FVC: Stat. sig. higher in non-users compared with VP group (p=0.001). FeNO: NS diff. between groups (p=0.156). PEF: NS diff. between groups (p=0.071).	
Singh et al., 2019, US (78)		n = 48 Healthy participants without chronic diseases or respiratory infections.	Vapers (n=22): exclusive VP users. Non-users (n=26): never users of tobacco products.	FEV1: NS diff between groups. FVC: NS diff between groups. FEV1/FVC: NS diff between groups.	8/20

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Mean age: 34.6, 56.3% females, 60.4% white, 18.8% African American, 14.6% Asian, 6.3% Hispanic, mean BMI: 26.1 kg/m^2.		PEF: NS diff between groups.	
Participants with asthma diagnosis					
Lappas et al., 2018, Greece (106)	Cross-over, Single use (A)	n = 54 Smokers, asthma (n=27): with mild asthma diagnosis Smokers (n=27): healthy smokers. Mean (SD) age: 23 (3.2), 38.9% females, mean (SD) BMI: 23.3 kg/m^2 (4).	Vaping (n=54): 10 puffs of 4 s each and 30 s inter-puff intervals on a cartridge VP (1.6 Ω resistance, 3.7 V battery voltage) with 46.13%/34.3% PG/VG vaping liquid, tobacco flavour and 1.18% nicotine strength. Non-use (n=54): sham use of the same VP for 5 minutes without the liquid and resistor coil.	Spirometry measures only taken at a screening session. FeNO measurements taken just before and after exposure to the two conditions. FEV1: stat. sig. higher in healthy smokers compared with smokers with asthma (p=0.002). FVC: NS diff. between healthy smokers compared with smokers with asthma (p=0.958). FEV1/FVC: stat. sig. higher in healthy smokers compared with smokers with asthma (p<0.001). PEF: stat. sig. higher in healthy smokers compared with smokers with asthma (p=0.034).	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				FeNO NS between healthy smokers and smokers with asthma at baseline. (0 min, 15 min, 30 min): stat. sig. decrease in both groups at 0 min after exposure compared with baseline (p<0.001). Stat. sig. decrease within smokers with asthma group at 15 minutes compared with baseline (p=0.013); NS diff. within healthy smokers' group. NS diff. within both groups at 30 minutes after vaping compared with baseline. NS differences in control session for both groups. IOS measures are reported in the narrative.	
Kotoulas et al., 2020, Greece (79)	Acute exposure, Single use (A)	n = 50 Smokers, asthma diagnosis (n=25): mean (SD) age: 40.6 (10.8), 48% females, mean (SD) BMI: 26 (5) kg/m2) Smokers, healthy (n=25); mean (SD)	Vaping (n=50): 10 puffs with 30 s inter-puff intervals for 5 minutes on a cartridge VP (NOBACCO, 1.2 Ω coil resistance) using 1-1.5 mL e-liquid of medium nicotine content.	FUs: 15 minutes after exposure, only FeNO was assessed 30 minutes after exposure. FEV1: NS diff. within and between groups after exposure. NS diff. between groups after exposure (p=0.628).	Low risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		age: 39.9 (10.2), 68% females, mean (SD) BMI: 26.5 (3.8) kg/m2).		FVC: NS diff. within and between groups after exposure. NS diff. between groups after exposure (p=0.480). FEV1/FVC: stat. sig. decrease within smokers with asthma group after exposure (p=0.040), NS diff. within healthy smokers' group (p=0.169). NS diff. between groups after exposure (p=0.677). PEF: stat. sig. decrease within smokers with asthma group after exposure (p<0.01), NS diff. within healthy smokers' group (p=0.321). NS diff. between groups after exposure (p=0.467). FeNO: stat. sig. increase within smokers with asthma (p<0.001) and stat. sig. decrease within healthy smokers (p<0.001). Stat. sig. diff. between groups after exposure (p<0.001). IOS measures are reported in the narrative.	

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
Solinas et al., 2020, Italy (99)	Longitudinal, 6 months (S-M)	n = 10 Outpatient asthma patients who had recently switched from smoking to VP use. Characteristics for this subset of the sample NR.	Vaping (n=10): ad libitum use of VP.	FUs at 3 & 6 months. Compliance: dual users were removed from the sample at BL and smoking history checked at FU. FEV1: NS diff. at both FUs compared with baseline. FVC: NS diff. at both FUs compared with baseline. FEV1/FVC: NS diff. at both FUs compared with baseline. PEF: NS diff. at both FUs compared with baseline. FeNO: NS diff. at both FUs compared with baseline. Measures of asthma changes were taken but we do not discuss these because of small sample size and absence of control group.	Moderate risk of bias
Aboelnaga et al., 2018, Egypt (85)	Cross-sectional	n = 130 Participants diagnosed with asthma but without a respiratory infection or asthma exacerbation within	Vapers (n=41): current VP use for ≥12 months. Smokers (n=41): current smokers having smoked >99 TC in lifetime.	FEV1: stat. sig. diff between groups with non-users > VP > smokers group (p<0.001) FVC: stat. sig. diff. between groups with non-users > smokers > VP group (p=0.02).	5/20

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		the last 2 months. Vapers (n=41): mean (SD) age: 30.4 (4.7), 53.7% females, mean (SD) BMI: 28.2 (6.5) kg/m2. Smokers (n=41): mean (SD) age: 29.5 (5.3), 46.3% females, mean (SD) BMI: 27.1 (5.8) kg/m2. Non-users (n=48): mean (SD) age: 30.3 (4.9), 54.2% females, mean (SD) BMI: 26.7 (6.8) kg/m2.	Non-users (n=48): NR.	FEV1/FVC: stat. sig. diff. between groups with non-users > VP & smokers' groups (p<0.001). NS diff. between VP and smokers' groups. PEF: stat. sig. diff. between groups with non-users > VP & smokers' groups (p<0.001). NS diff. between VP and smokers' groups. The Asthma Control Test (ACT) measures are reported in the narrative.	
Participants with COPD diagnosis					
Polosa et al., 2018, Italy (98)	Longitudinal, 36 months (L)	n = 44 COPD patients: Vapers (n=22): mean (SD) age: 65.2 (5.6), 13.6% females.	Vaping (n=20): ad lib use of own brand VP. Smoking (n=19): ad lib use of own brand TC.	FUs at 12 ± 1.5 months, 24 ± 2.5 months (retrospective) and 36 ± 3 months (prospective). Compliance: Mean (SD) CPD within vapers: 21.9 (4.5) at baseline, 2 (2.2) at 12 months, 1.6 (2) at 24 months and 1.5 (2.4) at 36 months.	Moderate risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Smokers (n=22): mean (SD) age: 66.5 (6.8), 18.2% females). (4/48 lost from BL: smokers 1 died, 1 moved away; vapers 2 relapsed to smoking)		13/22 were abstinent (self-reported & CO<7ppm); 9/22 dual users. Mean (SD) CPD within smokers: 20.8 (4.6) at baseline, 20.4 (3.7) at 12 months, 20.1 (5) at 24 months and 19.5 (3.8) at 36 months. FEV1: NS diff within groups at all FUs. NS diff between groups. NS diff within smoking-abstinent vapers at all FUs. FVC: NS diff within groups at all FUs. NS diff between groups. NS diff within smoking-abstinent vapers at all FUs. FEV1/FVC: NS diff. within VP group at all FUs.. Stat. sig. decrease compared with baseline within smokers' group at 12 (p=0.008) and 24 months (p=0.001), NS diff. at 36 months (p=0.664). NS diff between groups at 36 months FU. NS diff within smoking-abstinent vapers at all FUs. COPD exacerbation, GOLD staging and CAT score and 6MWD measures are reported in the	

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				narrative.	
Polosa et al., 2020, Italy (97)	Longitudinal, 60 months (L)	n = 39 COPD patients: Vapers (n=20): mean (SD) age: 66.9 (5.8), 15% females. Smokers (n=19): mean (SD) age: 65 (5.7), 15.8%. (9/48 lost from BL: smokers 1 died, 1 developed malignancy, 1 moved away, 2 quit smoking; vapers 4 relapsed to smoking or quit vaping)	Vaping (n=20): ad lib use of own brand VP. Smoking (n=19): ad lib use of own brand TC.	Earlier BL/FUs in Polosa et al. (98). FUs at 48 ± 3 months and 60 ± 3 months. Compliance: Mean (SD) CPD within vapers: 22.1 (4.7) at baseline, 2.2 (2.2) at 12 months, 1.8 (2) at 24 months, 1.4 (1.6) at 48 months and 1.4 (1.6) at 60 months. 9/20 were abstinent in exclusive vapers (self-reported & CO<7ppm); 11/20 dual users. Mean (SD) CPD within smokers: 20.2 (2.9) at baseline, 20.5 (3.6) at 12 months, 19.9 (5) at 24 months, 17.9 (3.9) at 48 months and 18.3 (3.4) at 60 months. FEV1: Stat. sig. decrease within VP group at 12 months (p=0.038) and stat. sig. increase at 48 (p=0.008) and 60 (p=0.001) months compared with baseline. NS diff within smoking group at all FUs. Stat. sig. diff. in overall between group p value from baseline (p=0.004) favouring vapers. Significant increase within smoking-	Moderate risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				abstinent vapers at 48- and 60-months FU (p =0.26 and p=0.003 respectively) but not dual users. FVC: Stat. sig. increase within VP group at 12 (p=0.046), 48 (p=0.008) and 60 (p=0.002) months compared with baseline. NS diff within smoking group at all FUs. Stat. sig. diff. in overall between group p value from baseline (p=0.016) favouring vapers. Significant increase within smoking-abstinent vapers at 48- and 60-months FU (p =0.034 and 0.003 respectively) but not dual users. FEV1/FVC: NS diff. within VP group at all FUs. Stat. sig. decrease within smoking group at 12 (p=0.008) and 24 (p=0.026) months compared with baseline. Stat. sig. diff. in overall between group p value from baseline (p=0.038) favouring vapers. NS difference within smoking-abstinent vapers at 48- and 60-months FU.	

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				COPD exacerbation, GOLD staging, CAT score and 6MWD measures are reported in the narrative.	
Participants with a mental health diagnosis					
Hickling et al., 2019, UK (61)	6 weeks (S-M)	n = 50 Smokers: with an established clinical diagnosis of schizophreniform, schizophrenia, schizoaffective disorder or bipolar disorder, or attending an early detection service in a high-risk state; daily smoking, confirmed via exhaled CO >5 ppm. Mean (SD) age: 39.0 (10.7), 24% females, 46% white, 42% black, 12% other ethnic group. Diagnosis: 54% schizophrenia, 20% schizoaffective disorder, 16% bipolar	Vaping (n=50): ad lib use of disposable VP (NJOY) with tobacco-flavoured 4.5% nicotine e-liquid. Participants were given free VP for 6 weeks, were encouraged to replace smoking with VP as much as possible and were informed about where they could purchase VP after initial 6 weeks.	Compliance: at 6 weeks, 37% had reduced CPD by ≥50% and 7% had stopped smoking. At 10 weeks, 26% had reduced CPD by ≥50% and 5% had stopped smoking. At 24 weeks, 25% (10 out of 40) had reduced CPD by ≥50% and 2.5% had stopped smoking. PEF: NS difference between baseline and week 6 (p=0.33), between weeks 6 and 10 (p=0.86), between weeks 10 and 24 (p=0.559). NS difference between participants who reduced CPD by >50% and non-reducers (p>0.05).	Serious risk of bias

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		disorder, 6% unspecified psychosis, 4% delusional disorder.			

Notes: A – acute exposure; CO – carbon monoxide; FeNO—fractional exhaled nitric oxide; FEV1—forced expiratory volume; FEV1/FVC—Tiffeneau-Pinelli index; FU—follow-up; FVC – forced vital capacity; L – long exposure; NS – non-significant; PEF – peak expiratory flow; RCT – randomised controlled trial; S-M—short-medium exposure; Stat. sig. diff. – statistically significant difference; TC – tobacco cigarette; VP – vaping product, nnVP – non-nicotine vaping product.

Synthesis of cell studies

Our search identified 47 in vitro studies that examined biological impact of exposure to vaping product aerosol or vaping product aerosol extract on various human airway cell types, including 3D airway epithelia cultures and co-culture systems (110-117); primary bronchial epithelial cells and cell lines (H292, Beas-2b, Calu-3, 16-HBE) (109, 118-142); alveolar type II epithelial cell line (A549) (140, 143-145); primary nasal epithelial cells (117, 134, 140); primary oral epithelial cells and cell lines (MOE1A, MOE1B, MSK-LEUK1) (146, 147); epithelial cancer cell lines from different head and neck regions (UM-SCC-1, WSU-HN6, and WSU-HN30) (148); oral and lung carcinoma cell lines (SCC-25, H1299, H441) (135, 149, 150); pulmonary microvascular endothelial cells (151); monocytes (U937) (136); gingival fibroblast cells (149, 152); pulmonary fibroblasts and pluripotent human embryonic stem cells (144); oral keratinocytes (153), squamous cell carcinoma (Fadu) and oral mucosal epithelial (Leuk-1) cell lines (154); and cells of animal origin (132, 133, 155) (appendices: table 7). Some of these studies are also considered in other health disease chapters where appropriate.

Seventeen of the above studies also reported the adverse effects following tobacco cigarette smoke (TC) exposure and 5 studies have examined exposure to HTP aerosol.

Two exposure methods have been used with the majority of studies exposing cells to vaping product, and TC and/or HTP aerosols, apart from 4 studies that extracted the aerosol or smoke into the culture medium before exposure (134, 136, 140, 148). The air-liquid interface (ALI) system has been utilised in 28 studies with the remaining studies using submerged cultures. There was considerable variation across exposure dose and duration and composition of e-liquids used to generate vaping product aerosol or extract.

Several studies have also been described in chapter 5 (flavours) (111, 112, 125, 127, 136-139, 143, 144, 146, 149) and chapter 9 (cancer) (111, 131, 136, 145, 148, 153). The comprehensive overview of the studies evaluating biological effects of vaping product exposure on airway cells is detailed in the appendices (table 7).

As it is becoming increasingly apparent that vaping product exposure induces molecular changes at the cellular level, most in vitro studies have focused on investigating toxicity at their primary site of exposure. Numerous airway cell types, including primary cells, established cell lines and co-cultures consisting of various cell types, have been used to evaluate cytotoxicity and cell viability following treatments with vaping product aerosol or vaping product aerosol extract at different time points and concentrations. Sixteen studies have reported significant increase in cytotoxicity or decrease in cell viability after exposure (114, 117-121, 125, 129-132, 143, 144, 146, 149, 150). Conversely, 5 studies showed that vaping product aerosol extract (134), vaping product and/or HTP aerosols (110, 111, 113, 115) yielded no impact on cellular toxicity, with the last 4 studies (110, 111, 113, 115)

disclosing funding from tobacco industry and/or vaping product manufacturers (see appendices: table 8).

When compared to the cytotoxic effects of cigarette smoke, 9 out of 11 studies agreed that vaping product-induced responses were substantially lower than that of TC exposure (111, 113-115, 117, 118, 120, 125, 146). Exposure to HTP aerosol has also produced a less marked effect compared to TC smoke, but to a higher extent than that of vaping product exposure (118, 120, 121). In contrast, one study found no effect on cytotoxicity in both TC and vaping product-treated cells (110). Similarly, Delaval and others (119) did not observe cytotoxicity after a single short exposure to TC smoke and aerosols generated from HTP and the 4th generation vaping product (Joyetech), although the 2nd generation vaping product (SmOKay) induced significantly higher cytotoxicity than those of HTP and controls (appendices: table 7).

Differential responses in cytotoxicity have been attributed to variations in nicotine concentration, flavourings and solvents (125, 129, 143, 144, 146, 149) and are partially discussed in chapter 6 (flavours). Several studies attempted to assess different exposure conditions, finding that the cytotoxic potential of the vaping product aerosols varies between device models (120, 125) and might increase as a function of atomizer's age (149), wattage settings increase (40W vs. 85W) (129), and resistance decrease from 1.5Ω to 0.25Ω (150) or 0.15Ω (131). In addition, variation in aerosol toxicity was observed across different human in vitro cell models. For example, embryonic stem cells appeared to be more sensitive to vaping product aerosols than pulmonary fibroblasts and lung epithelial A549 cells (144), while hepatocellular carcinoma Hep-G2 cells were found to be 30% more sensitive to the effect of e-liquid exposure than bronchial epithelium Beas-2b cells (125). Also, cell viability was decreased in response to vaping product aerosols in bronchial epithelial cell line Beas-2b, but not H292, and there was no significant effect on murine macrophage cell line (132). Another important aspect to be considered is that cytotoxicity and cell viability have been assessed using different in vitro assays which, although are effective in evaluating these outcomes, have different principles. While the neutral red uptake assay evaluates metabolic activity of the cells and MTT assay reflects their mitochondrial function, LDH assay is based on the principle that enzymes are released due to cell membrane damage and trypan blue assay is a good marker of membrane integrity. This may also explain some variability in findings between the studies. Therefore, due to differences in cell types, e-liquid composition, device characteristics and methodology, it is not surprising that the results are inconsistent.

In line with the cytotoxicity and cell viability data, many in vitro studies have reported vaping product -induced oxidative stress in different airway cell types. For example, Vasanthi Bathrinarayanan and others (114) investigated the effects of vaping product and TC exposures in the ALI model of co-culture of human bronchial epithelial cells with human pulmonary fibroblasts, finding that 180 puffs of vaping product aerosol increased levels of hydrogen peroxide (H_2O_2), a key metabolite in oxidative stress, but less than that

caused by 7 puffs of TC exposure, as compared to air-treated cells. Similarly, Tellez and others (146) demonstrated that exposure to vaping product aerosols of 10 different flavourings and nicotine concentrations induced oxidative stress levels up to 2.4-fold in at least one of 3 tested oral epithelial cell lines, with dose response seen for one vaping product aerosol across all cell lines. These effects were comparable in TC-exposed cells, while there was no significant change seen for unflavoured vaping product. Additionally, vaping product exposure has been associated with increased production of ROS (132, 136, 149), nitric oxide (132), mitochondrial superoxide (136), and changes in glutathione, cysteine and methionine metabolism (139). In contrast, 2 studies have reported no changes in the secretion of oxidative stress markers after vaping product exposure, whereas exposure to TC smoke led to increased levels of 8-isoprostane (111) and ROS (152). These discrepancies may arise from differences in vaping product aerosol constituents, cell types and exposure methods (such as dose, duration and aerosol generation).

The inflammatory response of different airway-derived cell types was assessed by measuring the release of inflammatory mediators in the culture medium following vaping product exposure and compared to what was observed in the control. Studies have reported significant increases in the levels of IL-6 and/or IL-8 (114, 117, 120, 122, 124, 129, 136, 145, 154), IL-1β and IL-10 (130), TNF-α and monocyte chemoattractant protein-1 (MCP-1) (117), prostaglandin E2 (136), soluble intercellular adhesion molecule-1, an endothelial protein induced upon inflammation (151), and significant decreases in secretion of MCP-1 and GROα (120). Three studies showed a very limited (decreased IL-12p40 only) (110) or no impact (111, 118) of vaping product exposure on cytokine levels, such as IL-1β, IL-6, IL-8, while TC exposure caused a strong inflammatory response. On the other hand, secretion of MCP-1 and GROα were decreased after exposure to vaping product aerosol in a manner similar to that of TC smoke, but after more intensive exposures (120). Interestingly, one study showed the opposite effects with decreased IL-6, IL-8, TNF-α and MCP-1 levels in TC-exposed cells but increased in vaping product - exposed cells compared to controls, which could result as a consequence of substantial decrease in cell viability in response to TC smoke (117). These different modulations in inflammatory mediators may again be down to the selection of cell type and exposure method.

Furthermore, to understand whether vaping product aerosol could affect the host defence activities of epithelial cells and, consequently, lead to respiratory tract infection, Herr and others (124) infected vaping product exposed human lung adenocarcinoma Calu3 cells with Pseudomonas aeruginosa. They found no effect on the bacterial count, barrier integrity and the expression of antimicrobial peptides after infection. In contrast, the corresponding amount of TC smoke negatively affected host defence and reduced barrier integrity (124). Another study exposed premalignant human oral mucosal epithelial Leuk-1 and malignant squamous cell carcinoma Fadu cell lines to vaping product aerosol and challenged by Porphyromonas gingivalis and Fusobacterium nucleatum, finding elevated

inflammatory response as evidenced by increased mRNA expression of TNF-α, IL-8, IFN-γ, IL-1β, and IL-6 with some being confirmed at the protein level, suggesting that vaping product aerosol can increase susceptibility to infection. In line with this, vaping product exposure in mouse bone marrow-derived primary macrophages led to impaired ability to clear dead cells and pathogens by efferocytosis and phagocytosis, and decreased bacterial clearance when challenged with Streptococcus Pneumonia (133). The authors also demonstrated that vaping product -exposed mouse macrophages exhibited apoptotic and inflammatory caspase–mediated cell death, while human and mouse lung epithelial cells exposed to the vaping product had increased apoptosis and secondary necrosis compared to controls.

The above outcomes were often accompanied by alterations in gene expression associated with oxidative stress and inflammatory pathways that have been described in the context of vaping product and TC exposures (112, 113, 116, 120, 124, 129, 131, 132).

Furthermore, several studies have investigated the effects of vaping product exposure on airway mucociliary function. Chung and others (126) indicated that airway surface liquid hydration and increased mucus viscosity of primary human bronchial epithelial cells at the ALI were negatively impacted by nicotine-containing vaping product. Two studies have reported that exposure to vaping product aerosol with and without nicotine stimulated expression of MUC5AC in human bronchial and nasal epithelial cells (109, 134). Given that MUC5AC plays an important role in mucin production, an abnormal increase could impair mucociliary transport system, which has been linked to increased risk of lung diseases, such as asthma and COPD, as well as altered innate immune response. Additionally, Clapp and others (127) demonstrated that cinnamaldehyde-flavoured vaping product aerosol rapidly yet transiently suppressed airway cilia motility of primary bronchial epithelial cells, which is essential in mucociliary clearance, as compared to PG/VG. Of note, vaping product aerosol had a modest inhibitory effect on mucous transport velocity one day post-exposure and vaping product aerosol sedimentation accounted for epithelial thickening in bullfrog palates (155).

Lastly, morphological changes in response to vaping product exposure have been observed in human primary nasal epithelial cells and engineered 3D nasal mucosa tissues, including a larger cell size and a faint nucleus compared to controls (117), but not in small airway epithelial cultures (112).

A few other vaping product-associated adverse effects have been reported in airway cell models as described in the appendices (table 7), but these are beyond the scope of this review.

Synthesis of animal studies

Our search identified 25 animal studies investigating the respiratory effects following vaping product exposure with 18 studies being conducted in a mouse model, including C57BL6 (156-165), Balb/c (166-170), Apoe-/- (171, 172), and B6C3F1 (173). Five studies utilised rats (174-178), with the remaining studies being conducted in guinea pigs (179) and sheep (126). Only 2 studies performed nose-only inhalation exposure (165, 176), while other studies exposed animals via whole-body inhalation, with durations of exposure ranging from a single dose to 6 months using various combinations of PG, VG, nicotine and flavourings or commercial e-liquids. Eleven studies compared the effects of vaping product aerosols and tobacco cigarette smoke, with one of these studies also including HTP products. Details for all the studies are presented in the appendices (table 6). Some of these studies are also considered in other health disease chapters where appropriate.

Given the extensive evidence on the detrimental consequences of tobacco smoking on the entire lung, respiratory effects of vaping product exposure have been widely investigated in animal models. Airway inflammation has been the most frequently assessed outcome with many studies reporting significant increases in inflammatory cellular influx, including macrophages, neutrophils, leukocytes, T-lymphocytes and dendritic cells as well as alterations in the levels of pro-inflammatory mediators in mouse bronchoalveolar lavage fluid (BALF) and/or lung tissues following vaping product exposure compared with air-controls (157-159, 164, 166, 167, 171). Some of the reported vaping product-induced effects were dependent upon nicotine concentration, specific flavourings, or mouse sex (157, 159, 164, 166). In addition, Chapman and others (168) demonstrated that flavoured vaping products without nicotine had a variable effect on inflammatory cells in BALF in a mouse model of allergic airways disease, while nicotine containing vaping products suppressed allergic airway inflammation. Furthermore, intrauterine vaping product exposure in mice, independent of nicotine levels, resulted in increased pro-inflammatory cytokines and altered inflammatory signalling pathways in both mother’s and offspring’s lungs (169). In contrast, one study did not identify significant changes in gene expression of pro-inflammatory cytokines in rats exposed to flavoured vaping products without nicotine (175). However, the authors found that manipulation of vaping product resistance influenced expression levels of chemokine (CCL3, CCL4, CSF2) encoding for macrophage inflammatory proteins, with the most marked changes in 0.25Ω group versus 1.5Ω.

Importantly, direct comparison with tobacco cigarette smoke exposure in mice suggested that chronic vaping product exposure induced little (165, 171) or no (162) changes in lung inflammatory responses, as opposed to tobacco cigarette smoke exposure. A similar trend was observed between vaping product and tobacco cigarette smoke exposed C57BL6 mice with minor changes in cytokine levels where only one out of 10 (163) and 2 out of 27 (161) cytokines were elevated in the larynx and lungs, respectively. Only one study showed equal increase in BALF cellularity, mainly because of macrophage influx, between tobacco-flavoured, nicotine-containing vaping product and tobacco cigarette smoke

exposed mice compared to air-controls (164). Lechasseur and others (170) examined the impact of unflavoured nicotine-free PG/VG exposure on normal lungs (vaping product vs. air-controls) and tobacco cigarette smoke-exposed lungs (dual vaping product and tobacco product vs. tobacco product) in Balb/c mice. Their results showed that PG/VG exposure alone or in combination with tobacco cigarette smoke reduced immune cell population in lungs and decreased expression of adhesion molecules that mediate cell recruitment (ICAM1, VCAM1) compared with corresponding air-controls and tobacco cigarette smoke exposure, with no major effects on BALF inflammation. The authors also concluded that PG/VG modified the effects of tobacco cigarette smoke exposure in dual-exposed mice on pulmonary expression of genes regulating the circadian molecular clock, such as arntl, nr1d1, nr1d2, per1, per2 and per3, although PG/VG alone induced mild changes in nr1d2 expression levels only. The earlier study by Khan and others (160) also showed differential expression and abundance of circadian molecular clock genes (clock and per2) and proteins (BMAL1 and PER2) in the mouse lungs following acute exposure to PG with nicotine compared to nicotine-free PG and air-control groups, pointing to the important role of nicotine in vaping product -induced effect on pulmonary circadian molecular clock disruption.

Alongside inflammatory and circadian changes, vaping product exposure has been associated with impaired lipid homeostasis and immunity in the lungs. Madison and others (162) demonstrated that C57BL/6 mice exposed to vaping products for 4 months, independent of nicotine, had impaired lung epithelial cell and macrophage function and appeared to be more vulnerable to viral infection with influenza A. This resulted in increased morbidity and mortality with persistent lung inflammation and tissue damage late in infection. Further, Szafran and others (158) identified that a 6-week vaping product exposure alone or with vanilla flavour in the same mouse strain significantly increased lung lipid-based immune mediators, 2-arachidonoylglycerol and 12-hydroxyeicosatetraenoic acid, that are believed to be pro- and anti-inflammatory. The authors also reported that vaping product exposure increased immunoglobulin 1 levels in BALF above those of the air-controls and dysregulated expression of lung genes related to immunotoxicity, with distinctive effects being produced by vanilla-flavoured vaping product aerosol as discussed in chapter 6 (flavours).

Vaping product exposure in mice has also been associated with increased markers of oxidative stress, such as myeloperoxidase (159), 8-oxodG (173), malondialdehyde and protein carbonyls (164) in BALF or lung tissues, as compared with air-controls. The effect of tobacco cigarette smoke exposure on malondialdehyde and protein carbonyls was equally pronounced (164), while levels of 8-oxodG in tobacco cigarette smoke exposed mice was also increased, but not significantly, most likely due to large inter-animal variability (173). Increases in plasma fibronectin, an indicator of tissue injury, were comparable between tobacco cigarette and vaping product exposure, independent of nicotine level (173). Similarly, studies in rats demonstrated pro-oxidative effects of vaping product exposure compared to air-controls, as evidenced by elevated malondialdehyde

content within the lung tissue (174), as well as enhanced activity of pulmonary xanthine oxidase, antioxidant and xenobiotic enzymes, ROS overproduction, increased carbonyl residues in pulmonary proteins and lipid peroxidation of erythrocytes, especially using lower vaping product resistance (175).

Many in vivo studies have addressed the effects of vaping product aerosol exposure on functional lung mechanics and airway hyper-responsiveness by evaluating airway response to increasing doses of aerosolised bronchoconstrictors such as methacholine. Szafran and others (158) demonstrated increased estimates of tidal and minute volumes, and tissue damping, an indicator of lung tissue resistance, in C57BL6 mice following 6-week exposure to nicotine-free vanilla-flavoured vaping product. A shorter vaping product exposure, independent of nicotine concentration, resulted in reduced basal inspiratory capacity and increased airway hyperresponsiveness in Balb/c mice (166). For comparison, a mouse model of allergic airways disease exhibited enhanced airway hyperresponsiveness along with increased lung collagen content in response to nicotine-free vaping product exposure with ‘Cinnacide’ and ‘Banana Pudding’ flavours, respectively, but not in the presence of nicotine (168). Given that there was no significant effect in other exposure groups, irrespective of nicotine concentration, the observed changes would appear to be linked to specific flavour additives rather than nicotine, but more research is needed to confirm this.

When compared with tobacco cigarette smoke, one study showed slightly impaired tissue elasticity, static compliance and airway resistance in C57BL6 mice after short-term 3-day nicotine-free vaping product exposure, but not tobacco smoke, while a more prolonged exposure of 4 weeks altered functional parameters in the tobacco cigarette group only (164). They also found an increase in airway hyperresponsiveness that was similar for both tobacco cigarette and nicotine-containing vaping product groups, however the authors did not report a long-term effect on airway hyperresponsiveness. Importantly, significant lung histological changes were induced by TC and not vaping product exposure (164). Three other studies that have compared the effects of vaping product and TC with air-exposure in mice reported minimal or no changes in lung function parameters and histological evaluation of airway tissues following vaping product exposure, while TC-exposed mice displayed increased incidence and/or severity of metaplasia, hyperplasia, emphysematous changes and other histopathological abnormalities (162, 165, 171). In one of these studies exposing mice to nicotine containing vaping product aerosol and TC smoke via nose-only inhalation for 3 weeks, transcriptional analysis revealed early perturbation of multiple biological pathways in the lungs associated with cell fate, cell proliferation, inflammatory and cell stress responses, and tissue repair (165). The most significant and consistent changes were obtained in response to TC with a very limited number of gene changes upon vaping product exposure. The results were aligned with those of a recent study conducted in ApoE-/- mice using whole-body exposure for up to 6 months, which reported TC-induced dysregulation of the lung transcriptome related to

same biological processes, with a milder impact observed in vaping product-exposed animals (171).

In a rat model, significant destructive changes in lung structure have been reported after 5-week vaping product exposure as reflected by reduced alveolar airspace area and pulmonary blood vessel count compared to air-controls, which was equal to the decrease observed in TC-exposed animals (178). Another study indicated that a 6-week exposure of rats to vaping product aerosol and TC smoke led to numerous lung morphological alterations accompanied by collagen deposition and inflammatory cells infiltration, but to a higher extent in the TC group (177). Interestingly, Kleinman and others (176) have reported initial findings from acute vaping product exposures in rats, showing histological changes in the airways when the device was operated at high power setting (70W vs. 60W) using nickel-chromium heating coil compared to stainless-steel atomizer heating element and air-controls. The observed alterations included pneumonitis in 2 out of 7 rats and multiple foci of pulmonary inflammatory cells in 4 out of 7 rats.

Two studies by the same research group indicated that acute (159) and sub-chronic (157) exposures to PG alone or with nicotine in mice altered myogenic, lipogenic and extracellular matrix (for example, collagen and fibronectin) markers at both mRNA and protein levels in a sex-dependent manner, suggesting a dysregulated repair response and ultimately remodelling of the lung tissue. Acute vaping product exposures have also led to increased protein levels of lung nicotine acetylcholine receptors alpha 3 and 7 (nAChRα3 and nAChRα7) compared to air-controls even in the absence of nicotine, pointing to the potential of PG alone to activate these nicotine receptors via other indirect mechanisms (159). Notably, sub-chronic vaping product exposures with nicotine resulted in up-regulation of angiotensin-converting enzyme 2 (ACE2), a SARS-Cov-2 Covid-19 receptor, whereas the deletion of nAChRα7 in mice showed down-regulation of ACE2, suggesting an important role of nAChRα7 in mediating ACE2 increase induced by nicotine-containing vaping product (157). Likewise, 2 recent studies observed increased lung ACE-2 expression of male C57BL/6 and Balb/c mice, but not female, exposed to nicotine containing vaping product aerosol (156, 166).

One study funded by Philip Morris International has examined the effects of vaping products, HTP aerosols and tobacco cigarette smoke on ceramide profile and functionally associated enzymes in mouse lung and plasma, finding that vaping product and HTP exposure, independent of nicotine or flavourings, did not induce significant changes, while tobacco cigarette smoke exposure did (172).

Lastly, vaping product-induced effects on lung function have been reported in guinea pigs and sheep. A single puff of nicotine-containing vaping product aerosol in anaesthetized guinea pigs induced a transient bronchoconstriction, most likely due to the activation of vagal bronchopulmonary C-fibers in response to stimulatory effects of nicotine (179). Exposure to nebulised vaping product aerosol with nicotine in sheep led to significant

reduction of tracheal mucus velocity, a marker of mucociliary clearance, despite a low but detectable plasma cotinine levels (126). Interestingly, the authors demonstrated that this vaping product-induced effect was reversed by pre-treatment with inhaled transient receptor potential ankyrin 1 (TRPA1) antagonist, indicating that TRPA1 receptor can mediate the effects of nicotine and vaping product aerosol.

10.4 Conclusions

In this chapter we reviewed the existing evidence on how vaping might cause or influence respiratory disease, one of the main causes of premature mortality and morbidity among smokers. This included summarising previous reports that have addressed this issue, and then presented findings from our systematic review of health risks and effects of vaping that are relevant to respiratory disease. Our systematic review aimed to assess the effects of exposure to vaping on biomarkers associated with the risk of health conditions and to assess the effect of vaping on disease outcomes in people with existing health conditions. Most studies examined ‘healthy’ participants, which we summarise first. We then summarise the studies which examined participants with respiratory conditions (asthma, COPD) and smokers with mental health conditions. We assessed both relative and absolute vaping risks associated with biomarkers of respiratory disease where the data were available (that is, between vapers and smokers, and between vapers and non-users), and where feasible included comparisons across different population groups.

Conclusions for biomarkers of exposure and biomarkers of potential harm cutting across common diseases are presented in chapter 7 and chapter 8. Several biomarkers of exposure are relevant to respiratory diseases. We identified conclusive evidence that under typical use conditions acute and short to medium exposure to most potential respiratory toxicants from vaping is significantly lower compared with smoking tobacco cigarettes, with substantial reductions in some biomarkers. For those respiratory toxicants that were assessed at long-term exposure, evidence was moderate that biomarkers of exposure are lower for vaping than smoking. For a few VOCs, such as formaldehyde and toluene, available evidence was inconclusive on the significant differences between vapers and smokers. However, one study suggested formaldehyde exposure might increase during compensatory puffing behaviour with lower nicotine strength e-liquids. In general, there were no significant differences between vapers and non-users except for acrylonitrile metabolite CNEMA for which the evidence suggested that vaping might increase exposure to acrylonitrile in absolute terms.

In relation to biomarkers of potential harm relevant to multiple diseases (including respiratory disease), such as 8-isoprostane and inflammation, evidence was mixed. This would therefore indicate that there was insufficient evidence from these biomarkers of potential harm whether vaping product use is associated with respiratory disease in humans.

We identified 25 studies (3 from the UK) that assessed other biomarkers of potential harm which were specifically related to respiratory disease in humans. Consistent with studies in other chapters, the included studies used a range of different designs and had varying quality or risk of bias. Studies included used a range of different definitions of vaping and smoking; for example, findings of some studies were confounded by treating vapers who smoke, occasional vapers or exclusive daily vapers as a uniform group or comparing occasional vapers with daily smokers. Hence findings need to be cautiously interpreted. Studies with more than one time point mostly explored acute exposure to vaping or followed-up participants for short to medium term, so we were unable to summarise findings on longer term vaping exposure. In line with our algorithm for selecting studies for meta-analyses, the lack of consistency in study designs, biomarker reporting, group definitions and exposure periods meant we were unable to carry out any meta-analyses. Of the 25 studies, 7 were relevant to our second research question about effects of vaping among people with existing health outcomes on disease outcomes: 4 assessed participants with asthma; 2 studies from the same longitudinal cohort, but with different follow-up rates, assessed participants with COPD; and one assessed participants with mental health disorders; these are summarised separately below.

All 25 studies included spirometry measures, a breath test used to assess airflow obstruction in the lungs and commonly used to detect respiratory diseases, but the different designs, groups and duration of exposure limited any conclusions that can be drawn. Overall, the findings indicated no acute, short to medium, or long-term detrimental effects for vapers, whereas a clear worsening of lung function was observed in one small study of vapers who switched back to smoking for 7 days. Eight studies assessed fractional exhaled nitric oxide (FeNO, a measure of nitric oxide in the breath and a marker of airway inflammation and asthma) and again involved different designs, groups, exposure duration limiting conclusions; there were mixed findings, but most reported no significant differences across the user groups. One study assessed impulse oscillometry which is an emerging respiratory diagnostic test and was suggestive of an effect of acute nicotine exposure on some lung function attributes among healthy occasional smokers but needs replication. Five imaging and bronchoscopy studies used a variety of different techniques and either assessed very short-term single-use exposure and/or were heavily confounded by including smokers (either of tobacco or marijuana) in the vaping groups. Overall, however, given the methodological differences, we concluded that there was insufficient evidence from spirometry, FeNO, impulse oscillometer, and bronchoscopy and imaging studies whether vaping has any impact on lung function after acute, short to medium and long-term exposure; and whether acute secondhand vaping had any effect on lung function.

In relation to our second research question, we first summarise our conclusions from the 4 studies with participants with asthma. Sample sizes were again generally very small, and the findings were inconclusive as to whether there are improvements in lung function and

respiratory symptoms among adult smokers with asthma who switch to vaping completely. There was limited evidence that vaping affects lung function among adults with asthma.

Turning to COPD, in the 2 longitudinal articles taken from the same cohort of COPD patients, a statistically significant improvement in some spirometry measurements were reported for the group who used vaping products compared with baseline but no significant differences in the group who smoked. However, only small numbers of participants were involved and the authors suggested larger studies were needed to confirm these findings. These findings indicate that there is limited evidence for reduction of chronic obstructive pulmonary disease (COPD) exacerbations among adult smokers with COPD who switch to vaping completely and continue vaping for up to 5 years.

Finally, one study with smokers with a mental health diagnosis who were encouraged to use a vaping product to reduce smoking reported no statistically significant changes in one spirometry measure but as most continued smoking, further research is needed with this population.

It is challenging to directly translate the findings from pre-clinical studies using human or animal cells or rodent models to any respiratory risks arising from vaping in humans. These pre-clinical studies commonly employ acute exposures sometimes over concentrated periods, and it is unclear whether the mechanisms or pathways to risk identified would be replicated in vapers. Further challenges arise because of the complex nature of vaping behaviour over time and the wide variety of different aerosols and products used. We identified 47 in vitro studies that examined biological impact of exposure to vaping product aerosol or vaping product aerosol extract on various human airway cell types and 25 animal studies investigating respiratory effects following vaping product exposure. Taking all the reviewed articles into consideration, the current available data contributes to the evidence that vaping product aerosol, to some extent, may cause airway-related adverse effects in cell and animal models, although it is inconclusive as to which constituents of the aerosol play key roles in the observed cellular and physiological effects.

Overall, while the literature has grown considerably since the NASEM report, the conclusions from that report are supported by our current review, as outlined above. The lack of consistency across the studies meant no meta-analyses of respiratory measures could be performed, limiting the conclusions that can be drawn. The limited evidence for improvements in COPD for adult smokers who switched to vaping reported in the NASEM report, has now been reported at the 5-year follow-up by the same study group; improvements seem mainly to be among those who switched to exclusive vaping. More studies have been carried out with people suffering from asthma but the different designs, diagnoses, measurements taken preclude any conclusions from being made.

10.5 Implications

Our quality assessments revealed most studies had some methodological concerns, and these should be addressed in future research as they limit interpretations of our findings. More research is needed, particularly in the UK, where we identified a dearth of studies.

As we previously mentioned, all studies we included had used very different methods. This included different designs, definitions of user groups (people who smoke, people who vape, people who smoke and vape, and people who do neither) and biomarkers. This likely resulted in discrepancies and variability in their findings.

As discussed in other chapters, the majority of studies exposed participants to brief sessions of vaping, and therefore cannot answer questions on long-term respiratory outcomes. Studies that assess people who have been vaping over long periods of time are therefore urgently needed. Findings from one long-term cohort of smokers who had switched to vaping at baseline are promising and should be replicated by other studies with larger numbers of participants.

More studies are needed that compare long-term former smokers who do and do not vape as well as studies comparing former smokers who vape with people who vape who have never smoked.

As many studies involve small numbers of participants, researchers should use other, less traditional ways to test their findings. This could include using a Bayes factor analysis to measure the strength of evidence. This is relevant to findings from most of the health biomarker studies included in this report.

For policy makers and practitioners, the limited evidence from our review for this chapter suggests that developing and implementing policies and interventions that support smokers to completely stop and switch to vaping is likely to slow down the development of respiratory diseases.

The choice of respiratory biomarkers also needs careful consideration. While some statistically significant changes in spirometry measures were observed, it is not clear whether these are too small to be clinically irrelevant, which raises the question of how useful spirometry measures are in relation to detecting any vaping risks, particularly among healthy smokers. This concern also applies to other biomarkers such as inflammatory changes. Additionally, the pathways between these biomarkers and increased risk of certain respiratory diseases still needs to be clearly mapped out with supportive evidence.

For human cell studies biologically relevant doses of nicotine or flavours that mimic exposure to vaping product aerosol emissions are needed.

Studying changes to the respiratory system is important as these might be the first signals of potential harms or (relative) benefits from vaping. Thus, seeking a global consensus on what measures should be studied and over what duration of exposure and follow-up, is urgently needed.

Finally, more studies are needed that assess the effects of vaping on people with pre-existing respiratory problems or diseases, both in comparison with no use of nicotine or tobacco and in comparison with smoking.

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11 Cardiovascular diseases

11.1 Introduction

Smoking and cardiovascular diseases

Tobacco smoke is a major preventable cause of cardiovascular diseases. The Global Burden of Disease Study estimates that for example 22% of deaths from coronary heart disease (one type of cardiovascular disease) are attributable to tobacco (1). Other main risk factors include dietary risks and environmental or occupational risks (1). In England, smoking caused around 12% of all deaths from cardiovascular diseases in 2020 (2).

Smoking causes coronary heart disease, stroke, atherosclerotic peripheral artery disease, and aortic aneurysm and early abdominal aortic atherosclerosis, and secondhand tobacco smoke causes coronary heart disease and stroke (3). There is a non-linear relationship between cigarettes per day and risk of coronary heart disease, with people smoking 5 or fewer cigarettes having 50% or higher the risk of people smoking 20 cigarettes per day (4). Benowitz indicated that inflammation, thrombogenesis, endothelial dysfunction, haemodynamic stress, arrythmogenesis, insulin resistance and lipid abnormalities are involved in accelerated atherosclerosis and acute cardiovascular events (4). Secondhand smoke also increases the risk of cardiovascular disease in people who do not smoke (4).

Smoking cessation reduces the risk of morbidity and mortality due to cardiovascular diseases. There are short-term benefits in terms of reduced risk and a continued decline in risk over the long term as time since cessation increases. In patients who are current smokers when for example diagnosed with coronary heart disease, the evidence is sufficient to infer a causal relationship between smoking cessation and reductions in all-cause mortality, deaths due to cardiac causes and sudden death and reduced risk of new and recurrent cardiac events (5).

Most of the cardiovascular health risk from smoking is due to inhalation of tobacco combustion products, such as oxidants, volatile organic compounds (VOCs), particulates and carbon monoxide. Nicotine may contribute to cardiovascular diseases by activating the sympathetic nervous system (increasing heart rate, blood pressure and myocardial work, and coronary artery constriction) and by affecting the lipid profile (4).

How vaping might affect cardiovascular health

The National Academies of Sciences, Engineering and Medicine (NASEM) report on the Public Health Consequences for E-Cigarettes (6) suggested several possible biological pathways for how vaping may theoretically affect the development of cardiovascular

disease. One main pathway suggested was that metals, oxidant chemicals and particulate matter could increase inflammation, platelet activation and thrombosis, endothelial dysfunction and atherosclerosis which in turn would increase the risk of myocardial ischemia and coronary heart disease via reduced myocardial blood, oxygen and nutrient supply and increased risk of coronary occlusion. A second suggested pathway was that nicotine would increase heart rate, blood pressure and myocardial contractility which would, via increased demand for oxygen and nutrients, increase the risk of myocardial ischemia and coronary heart disease. A major difference between vaping and smoking that NASEM highlighted was the absence of combustion chemicals such as polycyclic aromatic hydrocarbons and carbon monoxide when vaping (6).

11.2 Previous reports about effects of vaping on cardiovascular health and disease

Previous comprehensive reports on the effects of vaping on health come from NASEM in the US (6) and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in the UK, published in 2020 (7). COT is an independent scientific committee that provides advice to the Food Standards Agency, the Department of Health and Social Care and other government bodies on matters concerning the toxicity of chemicals. COT is an advisory non-departmental public body. In the methods chapter, we explain the rationale for summarising these reports.

The summary of reports and our systematic review below include human, cell, and animal studies. We give priority and most weight to human studies. We also include cell and animal studies for completeness but noted in chapter 2 (methods) their limitations and lack of transferability to humans, and comment on any specific notable limitations for individual studies below

Previous evidence reviews on vaping, commissioned by Public Health England (PHE)

Previous reviews in this series did not assess specific health outcomes. The 2018 report mentions that comparative risks of cardiovascular disease have not been quantified but are likely to be also substantially below the risks of smoking (8).

The National Academies of Sciences, Engineering and Medicine report on the Public Health Consequences of E-Cigarettes

NASEM reviewed 15 studies in humans to address effects of vaping on cardiovascular outcomes (6). Their conclusions were that there was:

- no available evidence whether or not vaping is associated with clinical cardiovascular outcomes (coronary heart disease, stroke, and peripheral artery disease) and subclinical atherosclerosis (carotid intima-media thickness and coronary artery calcification)

- substantial evidence that heart rate increases shortly after nicotine intake from vaping

- moderate evidence that diastolic blood pressure increases shortly after nicotine intake from vaping

- limited evidence that vaping is associated with a short-term increase in systolic blood pressure, changes in biomarkers of oxidative stress, increased endothelial dysfunction and arterial stiffness, and autonomic control

- insufficient evidence that vaping is associated with long-term changes in heart rate, blood pressure, and cardiac geometry and function

The Committee on Toxicity Statement on the potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems

COT aimed to review the potential human health effects and potential toxicity of vaping products with and without nicotine (7). Conclusions of COT with relevance to cardiovascular diseases include:

1. It is difficult to extrapolate from the findings of studies involving vaping product aerosol mixtures in experimental studies due to the wide range of variation in the mixtures tested, and devices and protocols used for aerosol production.

2. Nicotine has acute effects on the cardiovascular system, including increased heart rate and blood pressure. No data were identified regarding repeated or long-term inhalation exposure to nicotine per se in humans and data on longer term effects of nicotine exposure from vaping products were not available. Some evaluations have been made based on data from studies of nicotine replacement therapy (NRT) as an aid to quit smoking. On cardiovascular disease, these evaluations have concluded that studies are mostly of inadequate quality to draw clear conclusions but have not shown evidence of serious cardiovascular events.

3. There were no unexpected findings and case reports of adverse conditions that appeared to be related to vaping did not provide evidence for any cause-and-effect relationships above what would be expected from the inhalation of vapour containing nicotine.

4. In considering the comparison of vaping with smoking, the Committee concluded that the relative risk of adverse health effects would be expected to be substantially lower from

vaping. This risk reduction would occur if people who are already smoking switch to vaping, or if vaping is taken up instead of smoking. However, the reduction in risk would depend on the endpoint considered and there was some evidence that dual use could lead to increased risk compared with smoking only, depending on use patterns. Pharmacokinetic studies have indicated that systemic exposure to nicotine from the types of vaping products that have been studied to date is lower than or equivalent to that from smoking, but generally not higher. Therefore, any toxicological risks related to nicotine exposure would not be expected to be increased on switching from smoking to vaping.

5. Uptake of vaping by non-users of tobacco products is likely to be associated with some adverse health effects to which the user would not otherwise have been subject.

6. While there is currently no information that this is leading to adverse effects on human health, this is an important data gap.

7. Vaping is associated with some emissions into ambient air, including nicotine. For most health effects, the risks to bystanders will probably be low in conventional exposure scenarios, although pharmacological effects from exposure to nicotine in ambient air may occur in some individuals.

11.3 Findings from the systematic review

As described in chapter 2, the systematic review had 2 aims. In relation to cardiovascular diseases, they were:

1. What effect does vaping and secondhand exposure to vaping products have on biomarkers that are associated with the risk of cardiovascular diseases?

2. What are the effects of vaping among people with existing cardiovascular diseases on disease outcomes?

The remainder of this chapter will provide a summary of studies in humans that assessed biomarkers of exposure with relevance to cardiovascular diseases which were presented more fully in chapter 7. It will then summarise findings for biomarkers of potential harm associated with cardiovascular diseases, specifically markers of oxidative stress, inflammation and endothelial harm, again from studies in humans. These were fully presented in chapter 8. This will be followed by a summary of studies looking at cardiovascular outcomes such as heart rate and blood pressure.

Finally, findings from in vitro (cell) studies and in vivo (animal) studies with relevance to cardiovascular diseases will be summarised.

Biomarkers of exposure with relevance to cardiovascular diseases

In chapter 7, we report findings on biomarkers of exposure in detail. Here, we reiterate the summaries for nicotine, specific VOCs and carbon monoxide due to their relevance to cardiovascular diseases.

For nicotine, there was substantial variation across the 60 studies included in the review. Levels of nicotine and nicotine metabolites in participants using vaping products differed according to study design, definitions of vaping and smoking, biomarker and biosample used, and exposure duration. To assess relative exposures to nicotine between vaping and smoking, we were able to carry out 5 meta-analyses of nicotine and nicotine metabolites (one longitudinal, 4 cross-sectional) among at least weekly vapers and smokers. All found no significant differences across the groups. From the narrative summaries, evidence suggests that over time and with increased experience of vaping, users can derive similar levels of nicotine as they can from smoking cigarettes. Levels of nicotine metabolites varied with vaping device characteristics. To assess absolute exposures between vapers and non-users, we were able to carry out 4 meta-analyses of nicotine biomarkers which, as expected, showed significantly higher levels among vapers than non-users. In general findings from the narrative reviews were similar for absolute nicotine exposures. There were no discernible differences between adults and adolescent exposures to nicotine and its metabolites.

Twenty-four studies assessed VOCs. Again, there was considerable variation across the studies in terms of design, definitions of vaping and smoking, biomarker measurements and exposure duration. Here we discuss findings for VOCs with relevance to cardiovascular diseases (methods: table 3), namely acrolein (as measured by metabolites CEMA and 3-HPMA) and benzene (as measured by metabolites S-PMA and MU); none of the studies assessed propionaldehyde.

For acrolein, findings indicate that people who vape have lower levels than people who smoke and levels similar to people who do not vape or smoke. A meta-analysis of randomised controlled trials (RCTs) found that the geometric mean 3-HPMA level was on average 71% lower among vapers than among smokers. Longitudinal studies also showed statistically significantly lower average levels of 3-HPMA in vapers than smokers. Meta-analyses using data from cross-sectional studies to compare people who vaped with those who smoked found no statistically significant difference in CEMA levels and statistically significantly lower 3-HPMA levels (on average 45% lower) among those who vaped. Meta-analyses comparing people who vaped with people who did not smoke or vape found no statistically significant difference in CEMA or 3-HPMA levels. Other studies that could not be included in meta-analyses generally supported findings from the meta-analyses. Across cross-sectional studies that measured urinary biomarkers of acrolein, vapers' CEMA levels were approximately between 2% and 74%, and non-users' levels were approximately between 1% and 81% of CEMA levels among smokers. Across studies that measured

urinary 3-HPMA, vapers' levels were approximately between 17% and 62%, and non-users' levels were between 12% and 68% of 3-HPMA levels detected among smokers.

In meta-analyses, metabolites of benzene did not differ statistically significantly between people who vaped, smoked or were abstinent. Studies not included in meta-analyses found levels among people who vaped to be lower than among people who smoked and higher than among people who were abstinent. Across cross-sectional studies that measured the urinary benzene biomarker S-PMA, vapers' levels were approximately between 33% and 124% and non-users' levels were approximately between 13% and 123% of S-PMA levels detected among smokers. Vapers' levels of biomarker MU were approximately between 70% and 159% and non-users' levels were between 105% and 168% of those reported among smokers.

Ten cross-sectional studies assessed metals in blood or urine. All 10 assessed cadmium levels, 9 assessed lead and 2 arsenic. Overall, there was inconsistency between studies assessing levels of metals among vapers in comparison to smokers and non-users, with some finding higher, similar or lower levels in vapers compared with smokers or non-users. One study (36) reported that vapers' urinary arsenic levels were approximately 90%, and non-users' levels were approximately 73%, of arsenic levels detected among smokers. Levels of urinary cadmium among vapers were approximately between 48% and 104% and levels among non-users were between approximately 52% and 125% of cadmium levels detected among smokers. Some metals, in the case of cadmium, have a very long half-life and can be influenced by many environmental exposures. Hence, a history of smoking will greatly affect the levels of metals among ex-smokers who vape. Cross-sectional research has limited control over extraneous variables and past use.

Thirty-three studies assessed carbon monoxide exposure. As for other biomarkers, there was considerable heterogeneity across the studies and user definitions. To assess relative exposures between vaping and smoking, 2 meta-analyses were carried out. Both showed significantly lower blood carboxyhaemoglobin levels among vapers than smokers (on average 76% lower in RCTs and 63% lower in cross-over studies). We were unable to carry out any meta-analyses of exposures between vapers and non-users but some interventional studies suggested that exposure to carbon monoxide (CO) in smokers who completely switch to vaping product use might be reduced to levels similar to non-users.

Full details of the studies and findings on these biomarkers of exposure are presented in chapter 7.

Biomarkers of potential harm to health cutting across common diseases

We included studies that reported on vaping product use and associations with biomarkers of potential harm that cut across several diseases (see methods: table 3), specifically

markers of oxidative stress, inflammation, endothelial function and platelet function (no further markers categorised as ‘other’ in the methods chapter were reported). Chapter 8 presents findings on these from the systematic review.

Markers of oxidative stress with relevance to cardiovascular diseases reported in 22 studies identified in the systematic review were oxidised low-density lipoprotein (LDL), high-density lipoprotein (HDL), 8-isoprostane (8-iso-prostaglandin F2α) and soluble Nox2-derived peptide. Evidence on blood LDL levels was consistent across 8 studies of different design (including a meta-analysis of 2 cross-sectional studies), indicating no differences after acute and short-to-medium use of vaping products, smoking or non-use of tobacco and nicotine products. Blood HDL levels were similar between vaping product users, smokers and non-users in studies with smaller sample sizes (and in the meta-analyses we were able to perform) but were significantly higher among non-users in studies with larger sample sizes. Considering LDL and HDL associations with diet, physical activity and genetics, the current evidence does not indicate how vaping product use might affect LDL and HDL levels. Comparisons of levels of 8-isoprostane between vaping product, ‘dual use’, smoking and non-use groups after acute vaping or smoking exposures found mixed results. It has been suggested that participants’ longer past smoking history, older age and female gender might be associated with elevated 8-isoprostane levels (indicating oxidative stress). In general, evidence from the 9 included studies did not suggest strong associations between vaping and 8-isoprostane levels. Evidence on other oxidative stress biomarkers, including soluble Nox2-derived peptide, was limited, mixed and likely confounded by other factors, therefore further conclusions about vaping associations with these biomarkers cannot be made.

Twenty-five of the included studies reported on the association between vaping and inflammation biomarkers. However, heterogeneity of study designs, vaping and smoking definitions and methods for measuring biomarker levels preclude drawing clear conclusions about how vaping product use might compare with smoking or non-use in terms of inflammation. We were able to carry out meta-analyses for 2 cross-sectional studies for blood C-reactive protein (CRP). There were statistically significant lower blood CRP levels among vapers than smokers, and similar levels between vapers and non-users. Three other cross-sectional studies assessed blood CRP, observing similar findings for the comparisons between vapers and non-users, but not vapers and smokers, albeit with large variance within the latter 2 study groups. However, the one RCT that assessed CRP found no difference in changes between smokers randomised to vaping or continued smoking at 1-month follow-up and one non-randomised longitudinal study that assessed CRP after vaping reported significant increases among vapers.

Eleven studies reported on multiple endothelial function markers, as for oxidative stress and inflammation markers, they differed in study design, outcome measures and comparison groups. Many studies reported on changes in flow-mediated dilation (FMD) after acute or short-to-medium exposure to vaping product use. The available evidence

suggests that FMD tends to reduce (that is, worsen) after acute exposure to vaping products with and without nicotine, but a single RCT found that switching from smoking to vaping or even ‘dual use’ significantly improved FMD in a relatively short period of 4 weeks. Evidence from 2 cross-over and one non-randomised longitudinal study suggests that acute exposure to vaping might reduce the nitric oxide bioavailability similarly to acute smoking but also noted that past smoking history was an important confounder affecting the magnitude of change in nitric oxide bioavailability after acute exposure sessions. One cross-over and one acute exposure study reported significant increase in blood endothelial microvesicles among occasional smokers and non-smokers after acute exposure to nicotine vaping, and no change in after non-nicotine vaping. Evidence was inconsistent or inconclusive for changes in E-selectin and P-selectin after acute exposure to vaping, and there were no studies exploring changes in these endothelial function markers or in microvesicle activation after longer exposure to vaping product use. In addition, only a single cross-sectional study included a non-user group in endothelial function comparisons, therefore no conclusions could be drawn about endothelial function differences between vapers and non-users of tobacco and nicotine products.

We identified only 4 studies that reported on vaping associations with platelet function markers, and no clear conclusions could be made on how acute or longer-term vaping might affect platelet function in comparison to smoking or non-use of tobacco and nicotine products.

Full details of the studies and findings on these biomarkers of oxidative stress, inflammation, endothelial stress and platelet function are presented in chapter 8.

Biomarkers of potential harm with specific relevance to cardiovascular diseases

For cardiovascular outcomes, we identified 41 studies in humans to be included in the systematic review (9-49) (table 1).

Study characteristics

Among the 41 human studies, most (21 studies) were cross-over studies, mostly using single sessions of exposure for each condition, with only 2 using longer exposures (48 hours (30) and 5 days (13)). One study with a longitudinal component also had a cross-over component (25) and is therefore shown twice in table 1 and counted as cross-over and longitudinal in the following sections. Thirteen of the cross-over studies had been conducted in the US (9, 13, 14, 30-34, 38, 39, 42, 44, 50), 3 in Belgium (35-37), 2 in Germany (15, 41) and one each in Greece (25), Italy (40) and Sweden (43). The sample size for these studies ranged from 20 (9, 40) to 145 (31); between none (9) and 67% (15) were women and mean age ranged from 21 (44) to 38 years (36) with many reporting mean age in the 20s. Cross-over studies included participants with different smoking and

vaping behaviours: 8 included only smokers (9, 15, 25, 35, 37, 39, 40, 43), 2 included only ‘dual users’ (13, 30), 2 included vapers and ‘dual users’ (34, 38), one included only vapers (36) 3 only non-users (42, 44, 50) and 5 included a mix of groups (14, 31-33, 41).

We also included 6 studies that measured effects of acute exposure to vaping without cross-over. Two of the acute exposure studies included secondhand exposure (26, 27). Among the acute exposure studies, 5 had been conducted in the US (11, 26-29) and one in Germany (10). Sample sizes ranged from 16 (11) to 149 (27); the proportion of women ranged from 33% (29) to 54% (27) and mean ages were between 20 (29) and 35 (10). Four of the studies recruited both vapers and non-users (11, 26, 27, 29), one recruited smokers (10) and one recruited never smokers (28).

Six studies were cross-sectional studies of which 2 had been conducted in South Korea (45, 46), 2 in the US (20, 49) and one each in Russia (47) and Turkey (48), with between 31 (49) and 7505 (45) participants of whom 0% (46) to 36% (47) were women and ages ranged from 21 (47) to about 40 years (48). All of them included participants with a range of smoking and vaping behaviours.

There were 6 longitudinal studies with follow-up between one (25) and 42 months (23), 2 conducted in Greece (17, 25), 2 in the UK (12, 24) and 2 in Italy (23, 51). Sample sizes ranged from 21 (23) to 209 (24), with 20% (17) to 57% women (25) and mean ages between 27 (23) and 48 years (25, 51). At baseline, participants in 5 studies were smoking (12, 17, 24, 25, 51), the sixth (23) included vapers and matched non-users.

Finally, 3 RCTs (16, 21, 22) randomised smokers to vaping or continued smoking or a sham intervention and assessed outcomes at follow-up ranging from 2 weeks (22) to 3 months (21). Two RCTs took place in the US (21, 22), one in the UK (16); they included between 114 (16) to 263 participants (21), with 40% (22) to 66% female (16) and mean ages between 43 (22) and 47 years (21).

None of the 41 studies specifically recruited people with existing cardiovascular diseases and many studies excluded people with cardiovascular symptoms. Two studies included participants with mental health diagnosis (12, 51). One study was funded by a tobacco company (24) and one by a vaping product company which subsequently was bought by the tobacco industry (51); information on funding for all studies is included in the Appendix.

A range of outcome measures related to cardiovascular disease was used, most frequently heart rate (assessed in 31 studies) and blood pressure (30 studies). Pulse wave velocity (PWV, a measure of peripheral resistance, long-term studies can indicate changes in arterial stiffness) was assessed in 9 studies and oxygen saturation in 3 studies (table 1).

Using the algorithm described in the methods chapter (methods: table 6), we were able to select 10 studies to be included in meta-analyses of effects on heart rate or blood pressure.

Quality of studies and risk of bias

The risk of bias was assessed using different tools depending on the study design. Full results of the risk assessments are in the appendix, the summary scores are included in table 1.

Cross-over studies and RCTs were assessed using the Cochrane RoB2 tool which categorises the overall risk of bias as low, some concerns or high. Among the 21 cross-over studies (including the initial cross-over part of one longitudinal study), one (37) was rated at low risk of bias, and 2 at high risk of bias (25, 41) because of a risk of carry-over effects (25) and missing outcome data respectively (41). The remaining 18 studies were rated as having some concerns. All 3 RCTs were rated as having some concerns (16, 21, 22).

The studies measuring acute exposure and longitudinal studies were assessed using the ROBINS-I tool which categorises the overall risk as low, moderate, serious or critical. Out of the 6 acute exposure studies, one was rated at low risk (28), one at moderate risk (10) and 4 at serious risk (11, 26, 27, 29). The study by Kizhakke Puliyakote and others (11) was seen at risk of bias in classification of interventions. The studies by McClelland and others (26, 27, 29) were rated as at serious risk because of biases due to confounding; one additionally was seen at risk of bias due to deviations from the intended intervention (29). Of the 6 longitudinal studies, 4 were at moderate risk (17, 23, 24, 51) and 2 at serious risk due to risk of confounding (25) and risk of bias in selection of the reported result (12).

Quality of cross-sectional studies was assessed using Biocross; studies can score a maximum of 20 points with higher scores representing higher quality. Scores for the 6 included studies ranged from 5 (47) to 15 (48).

In the summary of study findings, we highlight studies at serious or high risk of bias when presenting outcomes.

Study findings

Results are presented by outcome measure, with results of meta-analyses followed by a narrative summary of the studies not included in meta-analysis. Details for all studies are presented in table 1.

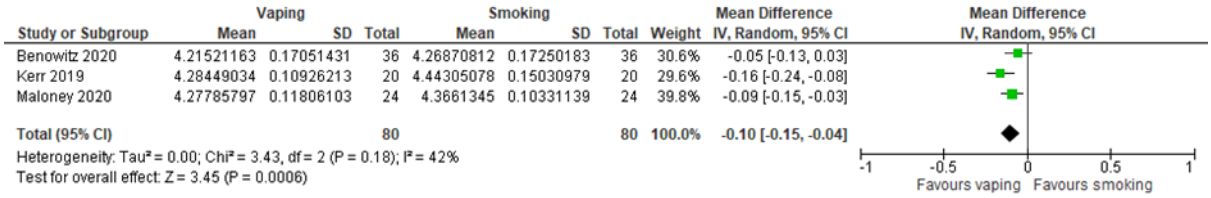
Heart rate

The measurement of heart rate was included in one of the RCTs (16), 5 longitudinal studies (12, 23-25, 51), 4 acute exposure studies (11, 26, 27, 29), 19 cross-over studies (9, 13-15, 25, 30-39, 41, 43, 44, 50) and 2 cross-sectional studies (20, 49). The RCT, 2 of the longitudinal studies (12, 24) and one of the cross-over studies (9) were from the UK. One study (30) assessed ambulatory heart rate with regular measurements over 24 hours, all others assessed heart rate within the experimental setting.

To compare acute effects of vaping and smoking on heart rate, we were able to meta-analyse 3 cross-over studies (figure 1) (9, 30, 39). All 3 were studies with some concerns regarding risk of bias. Heterogeneity was low and vaping was statistically significantly associated with lower heart rate than smoking (LMD: -0.10, 95% CI: -0.15, -0.04; 160 participants). The geometric mean heart rate after exposure to vaping was on average 9.5% (95% CI: 3.9%, 13.9%) lower than after exposure to smoking.

Among the studies that were not included in the meta-analysis, 2 studies (both at serious risk of bias) looking at effects of acute exposure reported an increase in heart rate after vaping among experienced vapers (11, 29). One acute exposure study at serious risk of bias reported a decrease in heart rate after vaping among people who were experienced vapers and an increase in non-users exposed to secondhand vapour (from nicotine vaping products) (27). Cross-over studies mostly reported an increase in heart rate after vaping (13-15, 31-34, 38, 50); one study (at high risk of bias) reported no significant change (25). One study (at high risk of bias) that compared different vaping conditions reported stronger increases for example with tank models compared with cartridges (41). Increases were sometimes similar to those seen after smoking (14, 31), and sometimes lower for vaping than for dual use or smoking (13, 15) consistent with the findings from the meta-analysis reported above.

Figure 1. Meta-analyses comparing heart rate for vaping and smoking in cross-over studies

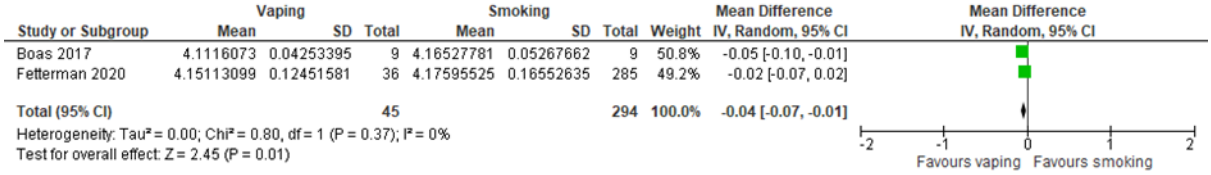


To compare longer-term effects in people who vaped and people who smoked, we were able to meta-analyse 2 cross-sectional studies (20, 49) (both quality score of 12/20). Again, heterogeneity was low and vaping was statistically significantly associated with lower heart rate than smoking (LMD: -0.04, 95% CI: -0.07, -0.01; 339 participants, figure 2). In relative terms, the geometric mean heart rate among people who vaped was on average 3.9% (95% CI: 1.0%, 6.8%) lower than among people who smoked.

Among the studies that were not included in the meta-analyses, the RCT found no significant differences in change in heart rate between smokers randomised to continued smoking or to attempt smoking cessation with vaping. This was at the 4-week follow-up when just under half of those randomised to vaping were not smoking (16). Similarly, a longitudinal study that followed up about half of the participants (smokers at baseline) found no significant change from baseline after 2 years of ad lib use of a vaping product (24). This was the same for the overall study population and participants who had been

abstinent from smoking (biochemically verified) for at least 80% of the completed study days. No statistically significant differences were also observed from baseline to one month follow-up in heart rate for any of the groups (those who continued to smoke, those who vaped and those who smoked and vaped) in another study at serious risk of bias (25). One of the 2 studies that examined smokers with mental health diagnosis who were encouraged to switch to ad libitum vaping product use did not find statistically significant heart rate changes at 6 weeks follow-up (11), while the other reported statistically significant reductions in heart rate at 12 weeks follow-up (51); most participants in both studies were concurrently smoking and using vaping products at follow-up.

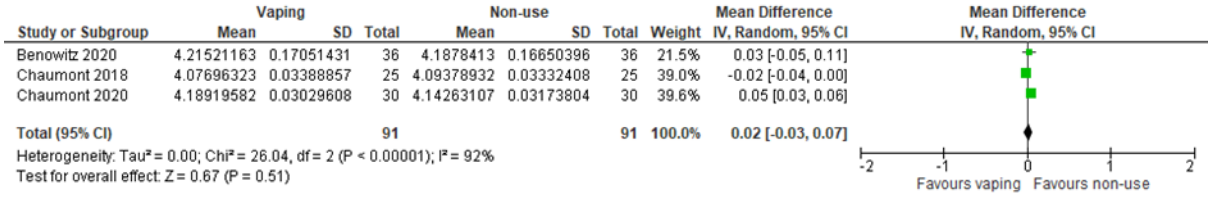
Figure 2. Meta-analyses comparing heart rate for vaping and smoking in cross-sectional studies



Three cross-over studies (30, 35, 36) could be included in a meta-analysis of heart rate after vaping compared with 48h abstinence or brief sham vaping (figure 3). All studies had some concerns related to risk of bias. Heterogeneity was low and differences were not statistically significant (LMD: 0.02, 95% CI: -0.03, -0.07; 182 participants).

Two further cross-over studies that could not be included in the meta-analysis assessed heart rate after exposure to vaping and sham vaping (37, 50). Both found no significant differences within groups after exposure but a higher heart rate after vaping compared with the sham condition. One acute exposure study (11) found no difference between groups after exposure, but a significant increase in heart rate after vaping. Another acute exposure study found a significant decrease in heart rate after vaping and no significant change in non-users' heart rate after 20 minutes of exposure to secondhand vaping and no significant difference in change between the 2 groups (26).

Figure 3. Meta-analyses comparing heart rate for vaping and non-use in cross-over studies

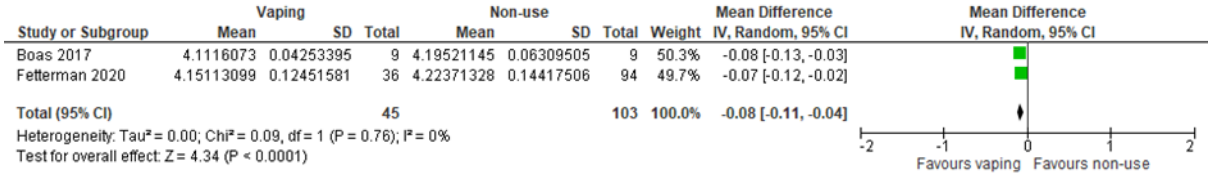


For longer-term associations with heart rate, we meta-analysed 2 cross-sectional studies (quality scores 12/20) (20, 49) to compare people who vaped and non-users.

Heterogeneity was low; people who vaped had statistically significantly lower heart rates than people who did not currently vape or smoke (LMD: -0.08, 95% CI: -0.11, -0.04; 148 participants, figure 4). This means that the heart rate of people who vaped was on average 7.7% (95% CI: 3.9, 10.4) lower than among people who did not vape or smoke.

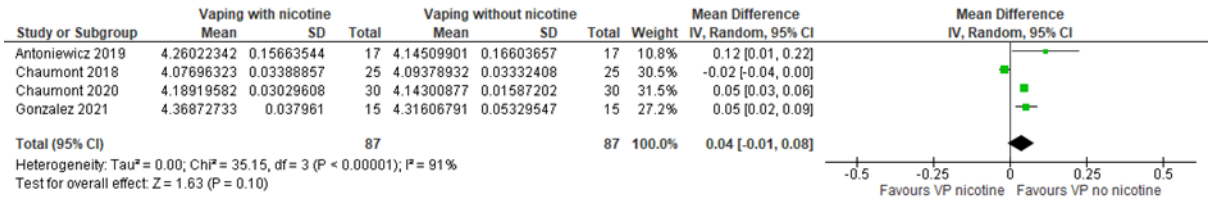
In one further cross-sectional study that could not be included in the meta-analysis, heart rates in those who vaped were higher than among those who did not vape or smoke (48). One longitudinal study (23) found no significant differences in heart rate between people who vaped and people who did not use any nicotine products when followed up after 12, 24 and 42 months.

Figure 4. Meta-analyses comparing heart rate for vaping and non-use in cross-sectional studies



We also ran a meta-analysis comparing acute heart rate after vaping nicotine and non-nicotine vaping. Four cross-over studies, all with some concerns regarding risk of bias, were included (35, 36, 43, 44). Heterogeneity was low and no statistically significant difference was detected (LMD: 0.04, 95% CI: -0.01, 0.08; 194 participants, figure 5).

Figure 5. Meta-analyses comparing heart rate for vaping and non-nicotine vaping in cross-over studies



Where other studies included non-nicotine vaping, most reported no change in heart rate after non-nicotine vaping (15, 25, 34) or a smaller increase when compared with nicotine vaping (31, 50). One cross-over study did not report the results for the non-nicotine exposure (39) other than that heart rate was significantly higher in the smokers' group compared with nicotine and non-nicotine vaping. In the RCT, there were no differences in change between groups randomised to nicotine or non-nicotine vaping (16). In a study examining secondhand vaping exposure to non-nicotine vaping (at serious risk of bias), there was no statistically significant change observed in heart rate, and no difference with users vaping non-nicotine products (26).

Summary of studies on heart rate

In a meta-analysis, we found lower heart rate after acute exposure to vaping than smoking. Other acute exposure studies not included in the meta-analyses also mostly found increases in heart rate after vaping similar or lower to those after smoking; one that compared different vaping devices found larger increases for tank models.

A meta-analysis comparing heart rate after acute exposure to vaping and abstinence detected no difference. Other studies comparing acute exposure to vaping with abstinence also found no difference between groups but an increase after exposure to vaping.

When comparing nicotine and non-nicotine vaping, a meta-analysis detected no differences in heart rate after acute exposure and other studies reported no or small increases after non-nicotine vaping.

In a meta-analysis of longer-term vaping and smoking, we also found lower heart rate in people who vaped. Other longer-term studies not included in the meta-analyses mostly found no differences between groups who vaped and smoked, however, those categorised as vapers often also smoked.

A meta-analysis of longer-term studies found that people who vaped had lower heart rate than people who did not vape or smoke. One further study found the opposite.

One longer-term study reported the same level of change in smokers switched to nicotine or non-nicotine vaping.

Only 2 small studies at serious risk of bias assessed secondhand exposure, precluding conclusions being drawn.

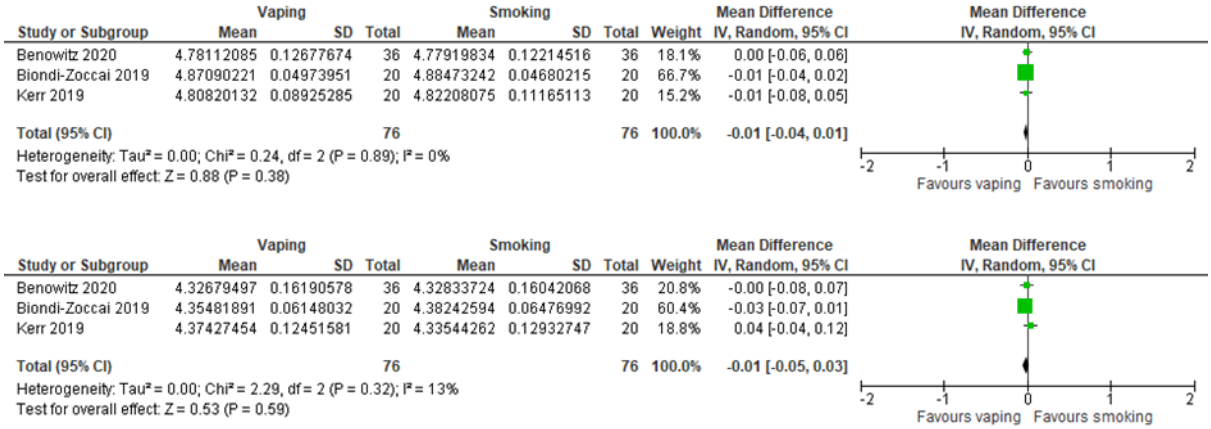
Blood pressure

Blood pressure was assessed in 2 RCTs (21, 22), all 6 longitudinal studies (12, 17, 23-25, 51), 3 acute exposure studies (26, 27, 29), 13 cross-over studies (9, 13, 15, 25, 30, 32, 35, 36, 40, 42-44, 50) and all 6 cross-sectional studies (20, 45-49). Two of the longitudinal studies (12, 24) and one of the cross-over studies (9) were from the UK. One study (30) assessed ambulatory blood pressure with regular measurements over 24 hours, all others assessed blood pressure within the experimental setting.

For acute changes in systolic and diastolic blood pressure, there were 3 cross-over studies (9, 30, 40) that allowed meta-analysis for the comparison of vaping and smoking. Heterogeneity was low and there was no evidence of difference (systolic blood pressure: LMD: -0.01, 95% CI: -0.04, 0.01; 152 participants; diastolic blood pressure: LMD: -0.01, 95% CI: -0.05, 0.03; 152 participants, figure 6).

Similar to the findings from the meta-analysis, 3 further cross-over studies reported no change in blood pressure after vaping exposure among people who usually smoked and/or vaped (13, 15, 25) (Cobb and others (13) and Ikonomidis and others (25) were at high risk of bias). Other cross-over studies reported an increase in blood pressure after vaping in people who smoked (43) and in people who usually vaped or were abstinent from smoking and nicotine (32). Two studies assessed blood pressure changes in people naive to smoking and vaping (42, 44); one reported no change (42), the other an increase in blood pressure after vaping (44). Two acute exposure studies that were both at serious risk of bias exposed people who usually vaped and non-users to vaping; one found no significant changes in vapers' blood pressure after exposure to non-nicotine vaping (26), the other found a difference due to a decrease in blood pressure in the non-user group after exposure while blood pressure among vapers remained unchanged (27).

Figure 6. Meta-analysis comparing systolic (top graph) and diastolic (bottom graph) blood pressure for vaping and smoking in cross-over studies

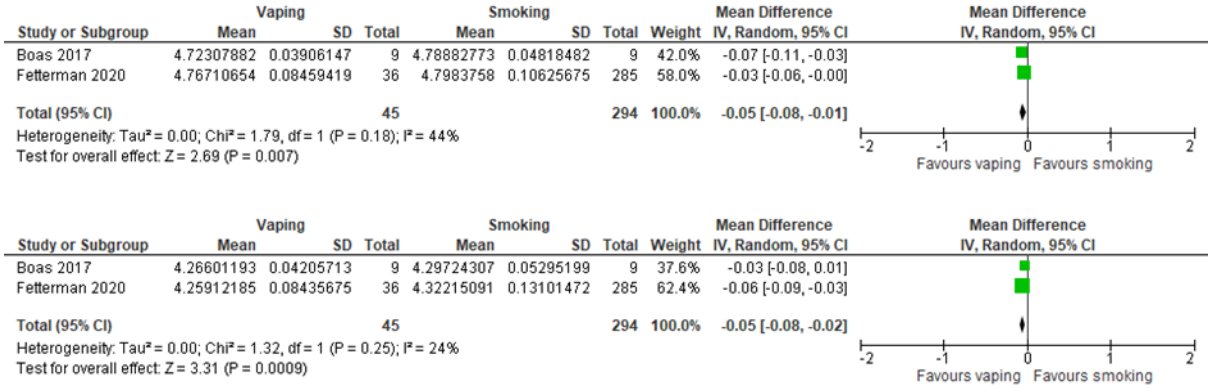


For longer-term changes, there were 2 cross-sectional studies (20, 49) that allowed meta-analysis for the comparison of vaping and smoking. Heterogeneity was low and people who vaped had statistically significantly lower systolic (LMD: -0.5, 95% CI: -0.08, -0.01; 339 participants) and diastolic blood pressure (LMD: -0.5, 95% CI: -0.08, -0.02; 339 participants) than people who smoked (figure 7). Expressed as geometric mean differences, people who vaped had on average 4.9% lower systolic (95% CI: 1.0, 7.7) and diastolic (95% CI: 2.0, 7.7) blood pressure than people who smoked.

Among the studies that were not included in the meta-analyses, 2 RCTs (21, 22) found no differences in blood pressure (or change in blood pressure) between smokers randomised to continued smoking or vaping at follow-up (although substantial proportions of each group had not achieved smoking abstinence). One longitudinal study reported no change from baseline among smokers who started using vaping products (24), both in the overall study population and those who had been abstinent from smoking on at least 80% of study days. Another longitudinal study reported a decrease in systolic blood pressure (but not diastolic) among smokers who switched to vaping or had started vaping (dual use) and no

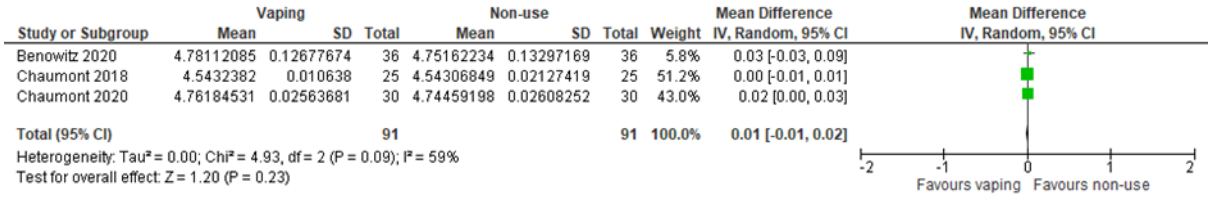
change among those who continued to smoke (25) (serious risk of bias). Of the 2 studies that explored changes in blood pressure after ad libitum use of vaping products among participants who were smoking and had a diagnosed mental health disorder, one did not find changes in systolic blood pressure after 6 weeks (12) (serious risk of bias) and another reported a decrease in systolic and diastolic blood pressure after 12 weeks of using vaping products (51) (moderate risk of bias).

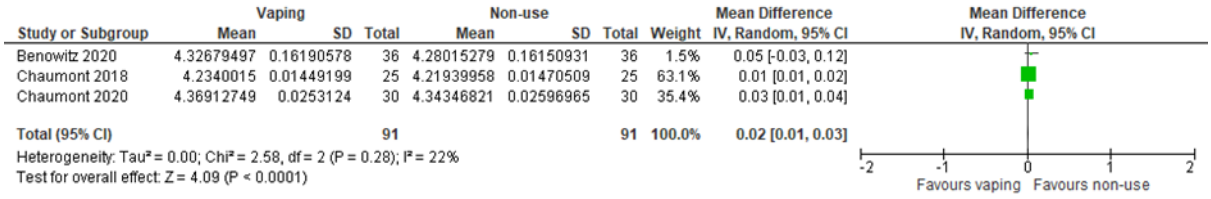
Figure 7. Meta-analysis comparing systolic (top graph) and diastolic (bottom graph) blood pressure for vaping and smoking in cross-sectional studies



Three cross-over studies (30, 35, 36) could be combined in meta-analyses comparing blood pressure after vaping and non-use. Heterogeneity was low and there was no difference between groups' systolic blood pressure (LMD: 0.01, 95% CI: -0.01, 0.02; 91 participants). For diastolic blood pressure, a statistically significant (LMD: 0.02, 95% CI: 0.01, 0.03; 91 participants, figure 8), but small difference was found (on average, diastolic blood pressure was 2.0% (95% CI:1.0, 3.0) higher after vaping). One other cross-over study found no significant differences in change in blood pressure after brief exposure to vaping or sham vaping (50) and another found no significant differences in blood pressure after 5 days of exposure to vaping or non-use (13).

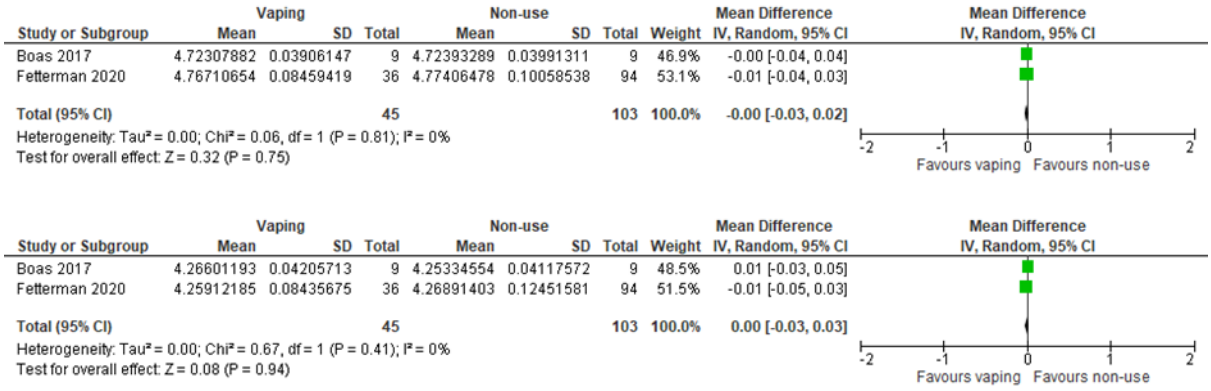
Figure 8. Meta-analysis comparing systolic (top graph) and diastolic (bottom graph) blood pressure for vaping and non-use in cross-over studies





A meta-analysis of 2 cross-sectional studies (20, 49) found no differences between people who vaped and people who did not vape or smoke (systolic: LMD: -0.00, 95% CI: -0.03, 0.02; 169 participants; diastolic: LMD: -0.00, 95% CI: -0.03, 0.03; 169 participants, figure 9). A longitudinal study found no statistically significant differences in systolic or diastolic blood pressure between people who vaped and those who used no nicotine product after 12, 24 and 42 months (23).

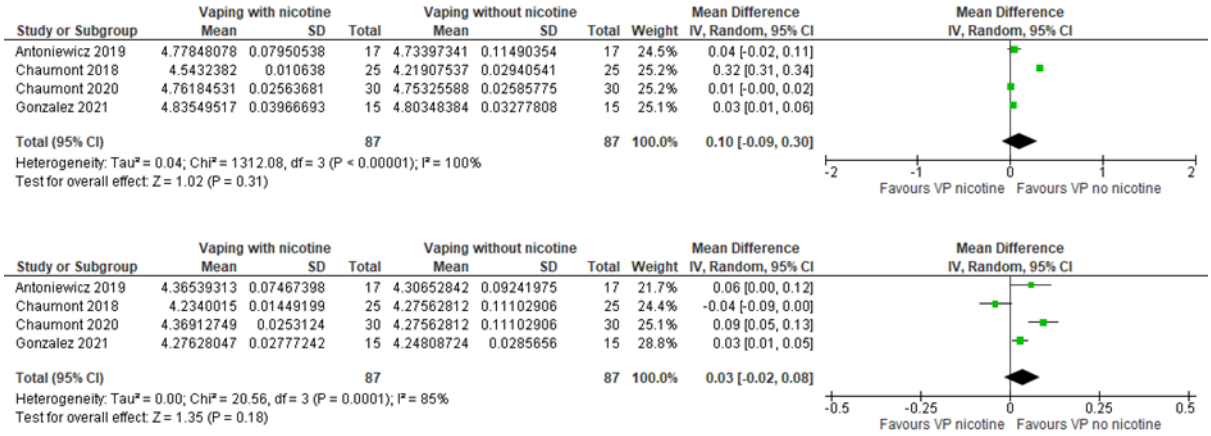
Figure 9. Meta-analysis comparing systolic (top graph) and diastolic (bottom graph) blood pressure for vaping and non-use in cross-sectional studies



The other cross-sectional studies found no differences in blood pressure between vapers and other groups (46-48); one found systolic blood pressure to be higher in smokers than in never smokers (45).

For a comparison of acute effects of vaping and non-nicotine vaping, 4 cross-over studies were meta-analysed (35, 36, 43, 44). Heterogeneity was low and no statistically significant differences were detected for systolic (LMD: 0.10, 95% CI: -0.09, 0.30; 174 participants, figure 8) or diastolic blood pressure (LMD: 0.03, 95% CI: -0.02, 0.08; 174 participants, figure 10).

Figure 10. Meta-analysis comparing systolic (top graph) and diastolic (bottom graph) blood pressure for vaping and non-nicotine vaping in cross-over studies



Where nicotine and non-nicotine vaping were compared in other cross-over studies, these generally found no statistically significant differences in blood pressure (25, 42, 50). However, one cross-over study reported a statistically significant decrease in diastolic blood pressure after non-nicotine vaping in people who usually smoked (15).

In one study (at serious risk of bias), there was a statistically significant decrease of systolic blood pressure within self-reported vapers when they vaped non-nicotine (but no significant change in diastolic blood pressure) and a statistically significant decrease of systolic blood pressure and diastolic blood pressure among the non-users after secondhand exposure to non-nicotine vaping (27).

Summary of studies on blood pressure

Meta-analyses comparing acute effects found no differences in blood pressure after acute exposure to vaping, smoking or non-use with the exception of a small difference between vaping and non-use for diastolic blood pressure. Studies that could not be meta-analysed found mixed results. A meta-analysis comparing acute effects of nicotine and non-nicotine vaping found no difference as did most other studies that could not be meta-analysed but included non-nicotine vaping. Only one small study at high risk of bias included secondhand exposure, so no conclusions can be drawn about effects on blood pressure.

Meta-analyses comparing groups with longer exposure found that people who vaped (presumably mostly former smokers) had lower blood pressure than people who smoked and that there was no difference between people who vaped and people who did not vape or smoke. Studies that could not be meta-analysed found mixed results.

Pulse wave velocity

PWV was assessed in 9 studies - one RCT (16), 2 longitudinal studies (17, 25), 2 acute exposure studies (10, 28), 4 cross-over studies (15, 25, 35, 43) and one cross-sectional

study (20). The RCT (16) was the only study from the UK. We were not able to combine studies into meta-analyses for PWV.

None of the studies included long-term changes to assess improvement or deterioration of arterial stiffness. The RCT reported no difference in change in PWV between groups at 4-week follow-up. One of the longitudinal studies reported a decrease in PWV in smokers after 4 months of vaping and an increase in those who continued to smoke (17) whereas the other study, which was at serious risk of bias, reported no differences at one month follow-up (25).

After acute exposure to vaping, PWV was often reported to increase among smokers (10, 15, 35, 43), with the exception of one study exposing smokers to vaping which was at serious risk of bias (25). In a cross-sectional study, no significant differences were seen between vapers, smokers, dual users, non-users (20).

After acute exposure to vaping among non-users in one study, no significant difference in PWV was reported (28).

All studies that assessed non-nicotine vaping reported no significant change in PWV after exposure (15, 25, 28, 35, 43).

Summary of studies on pulse wave velocity

In summary, pulse wave velocity may decrease after smokers have switched to vaping for a sustained period, however, no long-term follow-ups were reported. Pulse wave velocity generally increased after acute exposure to vaping nicotine, but not non-nicotine vaping.

Oxygen saturation

Oxygen saturation was assessed in 3 acute exposure studies, none from the UK and all at serious risk of bias (11, 26, 29). We were not able to combine studies into meta-analyses for oxygen saturation. One of the studies which was at serious risk of bias (29) reported a decrease after exposure to vaping, the other 2 reported no differences between groups or after exposure.

In summary, based on the very limited evidence available, no conclusion could be reached on the effect of vaping on oxygen saturation.

Table 1. Summary of studies in humans exploring associations between vaping products (VP) and cardiovascular health outcomes, arranged by study design

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
RCT					
George et al., 2019, United Kingdom (16)	4 weeks (S-M)	n = 114 Smokers: self-reported smoking of ≥15 TC per day for ≥2 years. Mean age 46.8, 65.8% females.	4-week ad libitum use of: Vaping (n=37): cartridge VP (Vapourlites), 16 mg/mL nicotine. Compliance defined as CO<6ppm. Vaping, no nicotine (n=37): cartridge VP (Vapourlites), 0 mg/mL nicotine. Compliance defined as CO<6ppm. Smoking (n=40): own-brand TC.	Compliance at 4 weeks: 19 (51.4%) in VP group had CO≥6 ppm 19 (51.4%) in nnVP group had CO≥6 ppm HR: NS diff. in change between the three arms at 4-week FU. PWV: NS diff. in change between the three arms at 4-week FU.	Some concerns
Pulvers et al., 2020, US (22)	6 weeks (S-M)	n = 186 Smokers: self-reported smoked ≥5 TC per day on ≥25 days of the past 30 days, smoked for ≥6 past months, had expired CO>5 ppm. Mean (SD) age: 43.3 (12.5),	Ad libitum use of: Vaping (n=125): pod VP (JUUL) with 5% strength nicotine salts and menthol (35.2%), mango (28%), mint (19.2%) or tobacco (17.6%) flavours at baseline.	FUs at 2 & 6 weeks. Compliance not enforced. SBP: NS diff. within VP, smoking and between groups at 2 and 6 weeks FUs. DBP: NS diff. within VP,	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		40.3% females, 49.5% African American, 50.5% of Latinx ethnicity.	Smoking (n=61): own brand TC.	smoking and between groups at 2 and 6 weeks FUs.	
Veldheer et al., 2019, US (21)	3 months (S-M)	n = 263 Smokers: on average 18.4 CPD for >1 years. Mean age 47, 60% females.	Ad libitum use of: Vaping (n=191): a tank VP (3.3 volts, 1000 mAh battery, 1.5 Ω dual coil) with 70%/30% PG/VG and 36, 8 or 0 mg/mL nicotine strength e-liquid. Other (n=72): a plastic cigarette-like tube that emits no vapour.	FUs at 1 & 3 months. Compliance: at 3-month FU, 69.2% of all participants dually used TC and assigned product, 26.6% exclusively smoked and 2.7% (n=6 VP & n=1 the TC substitute) exclusively used the assigned product; 1.1% (n=3) stopped smoking and using the assigned product. Analysis adjusted for age, race, gender, education, CPD, follow up visit, group, frequency and days of product use. SBP: NS diff. in changes between VP or TC substitute groups at month 1 & month 3. DBP: NS diff. in changes between VP or TC substitute groups at month 1 & month 3.	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
Longitudinal					
Caponnetto et al., 2021, Italy (51)	12 weeks (S-M)	n = 40 Smokers: adult outpatients at psychiatric clinics, smoking >19 CPD, not intending to reduce or stop smoking, having a schizophrenia spectrum disorder diagnosis without a recent exacerbation. Mean (SD) age: 48.3 (12.1), 35% females, 100% white Caucasian, mean (SD) age onset of schizophrenia spectrum disorders: 21.9 (2.8).	Vaping (n=40): ad lib use of pod VP (JUUL, 200 mAh integrated battery) with Virginia tobacco flavour 5% nicotine salt pods. Participants informed to use the VP as much as they like with free pods for 12 weeks.	Compliance: at weeks12 & 24, 37 out of 40 (92.5%) were followed-up. Mean (SD) CPD: baseline—28 (9.1), week 12—6.4 (6.9), week 24—6.9 (6.8). HR: stat. sig. decrease at week 12 compared with baseline (p<0.0001). SBP: stat. sig. decrease at week 12 compared with baseline (p<0.0001). DBP: stat. sig. decrease at week 12 compared with baseline (p<0.0001).	Moderate
Hickling et al., 2019, UK (12)	6 weeks (S-M)	n = 50 Smokers: with an established clinical diagnosis of schizophreniform, schizophrenia, schizoaffective disorder or bipolar disorder, or attending an early detection service in a high-risk state; daily smoking, confirmed via exhaled CO >5 ppm.	Vaping (n=50): ad lib use of disposable VP (NJOY) with tobacco-flavoured 4.5% nicotine e-liquid. Participants were given free VP for 6 weeks, were encouraged to replace smoking with VP as much as possible and were informed about where they could purchase VP after initial 6 weeks.	Compliance: at 6 weeks, 37% had reduced CPD by ≥50% and 7% had stopped smoking. At 10 weeks, 26% had reduced CPD by ≥50% and 5% had stopped smoking. At 24 weeks, 25% (10 out of 40) had reduced CPD by ≥50% and 2.5% had stopped smoking. HR: NS difference between	Serious

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Mean (SD) age: 39.0 (10.7), 24% females, 46% white, 42% black, 12% other ethnic group. Diagnosis: 54% schizophrenia, 20% schizoaffective disorder, 16% bipolar disorder, 6% unspecified psychosis, 4% delusional disorder.		baseline (mean (SD): 80.6 (16)) and week 6 (82 (15.8), n=46). SBP: NS difference between baseline (120 (15.3)) and week 6 (121 (16.1), n=46).	
Ikonomidis et al., 2020, Greece (17)	4 months (S-M)	n = 40 Self-reported smokers of mean 25.9 CPD. Mean (SD) age 44.8 (11.3), 80% females.	4-month ad libitum use of: Vaping (n=20): tank VP (NOBACCO eGo Epsilon BDC), 4.5% non-specified flavouring, 74.3% to 20% PG/VG ratio, 12 mg/mL nicotine Smoking: own-brand TC.	Compliance at 4 months: all VP group had eCO <10 ppm, 5/20 in VP group self-reported using 3-4 CPD. SBP: NS stat. diff in VP (p=0.949) and smoking (p=0.855) groups. DBP: NS diff in VP (p=0.641) and smoking (p=0.267) groups. PWV: Stat. sig. decrease in VP (p=0.047). Stat. sig. increase in smoking (p=0.028).	Moderate

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
Ikonomidis et al., 2018, Greece (25)	1 month (S-M)	n = 70 Smokers attending hospital's smoking cessation unit. Additional group of smokers (n=20) was a control group for FU at 1 month. Mean (SD) age: 48 (5), 56% females.	Vaping (n=42): ad lib use of a VP with 12 mg/mL nicotine. Dual use (n=24): ad lib use of the VP and own-brand TC. Smoking (n=20): ad lib smoking of own-brand TC.	Compliance at 1 month FU: self-reported mean (SD) CPD: Vapers: 0; Dual users: 5 (4); Smokers: 24 (7.1). HR: NS diff. within all groups at 1 month FU. SBP: stat. sig. decrease within vapers (p=0.03) and dual users' (p=0.04) groups at 1 month FU compared with baseline. NS diff. within smokers' group (p=0.5) at 1 month FU. DBP: NS diff. within all groups at 1 month FU compared with baseline. Arterial stiffness (augmentation index corrected for HR, AIx75): stat. sig. decrease within vapers (p=0.001) and dual users' (p=0.01) groups at 1 month FU compared with baseline.	Serious

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				NS diff. within smokers' group (p=0.4) at 1 month FU. PWV (carotid-femoral): NS diff. within all groups at 1 month FU.	
Polosa et al., 2017, Italy (23)	42 months (L)	n = 31 Vapers (n=16): self-reported smoked <100 TC in lifetime and were using a VP for ≥3 months. Mean (SD) age: 26.6 (6), 33.3% females. Non-users (n=15): age- and sex-matched non-users of tobacco and nicotine products. Mean (SD) age: 27.8 (5.2), 33.3% females.	42 months ad lib use of: Vaping (n=9): own-brand tank type VP with 0% (3/9), 0.9% (2/9), 1.2% (2/9), 1.6% (1/9) and 1.8% (1/9) nicotine strength vaping liquid with tobacco (7/9), mint (1/9) or fruit (1/9) flavours. Non-use of nicotine products (n=12).	FUs at 12, 24 and 42 months. Compliance at 42 months: 9/16 (56.3%) in VP group, 12/15 (80%) in non-users group. HR: NS diff. between groups (p=0.15) at all FUs. SBP: NS diff. between groups (p=0.82) at all FUs. DBP: NS diff. between groups (p=0.5) at all FUs.	Moderate
Walele et al., 2018, UK (24)	24 months (L)	n = 209 Smokers: self-reported smoking of 5-30 TC per day for ≥1 year. Mean (SD) age among those who switched	24 months ad lib use of: Vaping (n=209): cartridge VP (Puritane) with 1.6% nicotine strength, 67.5%/30% PG/VG vaping liquid with tobacco or	FUs at 1, 3, 6, 12, 18 and 24 months. Compliance: 102/209 (48.8%) followed-up at 24 months and were abstinent from smoking cigarettes for ≥80% of the study days.	Moderate

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		(n=109): 38.7 (10.2), 44.1% females, mean (SD) BMI: 26.2 (4).	menthol flavour.	HR: NS diff. at all FU points compared with baseline SBP: NS diff. at all FU points compared with baseline. DBP: NS diff. at all FU points compared with baseline.	
Acute exposure					
Caporale et al., 2019, US (28)	Single use (A)	n = 31 Non-users: healthy, young 18-35 years old with no history of smoking. Mean (SD) age: 24.3 (4.3), 45.2% females, mean (SD) BMI: 23 (2.4).	Vaping (n=31): 16 3-seconds puffs of a disposable VP (Epuffer, 3.7 V) with 70%/30% PG/VG, 15% flavour dilution (flavour NR) and 0 mg/mL nicotine e-liquid.	PWV (aortic arch): NS diff. after exposure (p=0.65).	Low
Kizhakke Puliyakote et al., 2021, US (11)	Single use (A)	n = 16 Vapers (n=9): self-reported VP use for >1 year, vaping daily. Mean (SD) age: 23 (5), 42.9% females, mean (SD) BMI: 25 (5).	Vaping (n=9): single ad lib use of disposable VP (Puff Bar) with 50 mg/mL nicotine strength nicotine salts. Non-use (n=7): no intervention.	HR: NS diff. between groups (p=0.2). Stat. sig. increase within VP groups after exposure compared with baseline (p=0.0005). %O2 saturation: NS diff. between groups (p=0.9).	Serious

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Non-users (self-reported not smoking or vaping, n=7). Mean (SD) age: 21 (2), 33.3% females, mean (SD) BMI: 24 (4).		NS diff. within VP groups after exposure compared with baseline (p=0.1).	
Kuntic et al., 2020, Germany (10)	Single use (A)	n = 20 Smokers: healthy, smoking on average 14 CPD and having 11.6 pack-years. Mean (SD) age: 34.7 (10.2), 50% females, mean (SD) BMI: 26.8 (3.9).	Vaping (n=20): 40 puffs with 30-second inter-puff interval for 20 minutes of a tank VP (Joytech eGo C) with tobacco flavoured, 18 mg/mL nicotine strength e-liquid.	PWV: stat. sig. increase during (p=0.0084) and 15 minutes after exposure (p<0.0001) compared with baseline.	Moderate
McClelland et al., 2020, US (29)	Single use (A)	n = 24 Self-reported: Vapers (n=12). Non-vapers (n=12). Mean (SD) age: 19.6 (0.9), 33.3% females, 54.2% white, 20.8% black, 25% of mixed or other ethnicity.	Vaping (n=12): ad lib use of own brand VP for 20 minutes. Non-vapers (n=12): only baseline measures collected.	HR: NS diff. between groups at baseline (p=0.27). Stat. sig. increase in VP group 20 minutes after exposure (p=0.05). SBP: stat. sig. higher in VP group compared with non-vapers' group at baseline (p=0.03). NS change in VP group 20 minutes after exposure (p=0.56). DBP: NS diff. between groups at baseline (p=0.80).	Serious

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				NS change in VP group 20 minutes after exposure (p=0.64). %O2 saturation: NS diff. between groups at baseline (p=0.41) Stat. sig. decreased in VP group 20 minutes after exposure (p=0.01).	
McClelland et al., 2021, US (27)	Single use (A)	n = 149 Self-reported: Vapers (n=76). Non-users (n=73). Mean (SD) age: 22.1 (7.3), 53.7% females.	Vapers and non-users were in the same 13.4 m2 room (first-hand and second-hand exposure). Vapers (n=76): ad lib use of a pod VP (JUUL) with mint-flavoured and 5% nicotine strength e-liquid for 20 minutes. Non-users (n=73): exposure to the same VP aerosol for 20 minutes.	Covariates: age, gender, present health, recreational drug use, use of cigarettes or alcohol, mental health treatment, presence of a lung, oral or cardiac disease. HR: NS diff. within groups after exposure. Interaction effect between groups after exposure (decrease in VP group, increase in non-users group, p=0.03). SBP: Stat. sig. diff. pre-post (repeated measures) main effect and interaction (p=0.02), due to decrease	Serious

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				in non-user group	
McClelland et al., 2020, US (26)	Single use (A)	n = 148 Self-reported: Vapers (n=73). Non-users (n=75). Mean (SD) age: 23.2 (9.2), 53.4% females, 75.5% white, 19.6% not white ethnicity.	Vapers and non-users were sat next to each other (first- and secondhand exposure). Vapers (n=73): ad lib use of a VP (Sorin) with 70%/30% PG/VG mix without nicotine or flavourings for 20 minutes. Non-users (n=75): exposure to the same VP aerosol for 20 minutes.	HR: stat. sig. decrease within vapers' group after exposure (p=0.022). NS change within non-users' group (p=0.124). NS diff. in change after exposure between VP and non-users' groups (p=0.585). SBP: stat. sig. decrease within vapers' (p=0.001) and non-users' (p<0.001) group after exposure. NS diff. in change after exposure between VP and non-users' groups (p=0.702). DBP: NS change within vapers' group after exposure (p=0.457). Stat. sig. decrease within non-users' group (p=0.010). NS diff. in change after exposure between VP and non-users' groups (p=0.168).	Serious

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				%O2 saturation: NS changes within VP (p=0.874) and non-users' group (p=0.177) after exposure. NS diff. in change after exposure between VP and non-users' groups (p=0.406).	
Cross-over					
Antoniewicz et al., 2019, Sweden (43)	Single use (A)	n = 15 Smokers: self-reported healthy occasional smokers of ≤10 TC per month. Mean (SD) age: 26 (3), 60% females.	Cross-over conditions separated by 1 week. Vaping, nicotine (n=15): 30 3-second puffs across 30 minutes of modular VP (32 W, 0.2Ω, dual coil atomizer), 49.5%/44.4%/5% PG/VG/Ethanol, 19mg/mL nicotine. Vaping, non-nicotine (n=15): same puffing regime on the same VP with 0mg/mL nicotine.	HR: stat. sig. increase within VP group in first 10-20 minutes (p=.015). NS diff within nnVP group. Stat. sig higher rate in VP compared with nnVP group (p<.001). SBP: stat. sig. increase within VP and nnVP groups in first 10 minutes (p<.001). NS diff. between groups (p=.227) DBP: stat. sig. increase within VP and nnVP groups in first 10 minutes (p<.001). NS diff. between groups	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				(p=.062) PWV: stat. sig. increase within VP in first 10-20 minutes (p=0.001), NS change within nnVP group. Stat. sig. difference between groups (p=.037).	
Benowitz et al., 2020, US (30)	48 hours (A)	n = 36 Dual users who used a VP ≥15 days and smoked ≥5 CPD over the past 30 days. A salivary cotinine level of ≥50 ng/mL. Mean (SD) age: 35.4 (11.7), 22% females, 61% mixed ethnicity, 14% white, 11% Latin, 8% Black, 6% Asian.	48-hour cross-over conditions in confinement: Vaping (n=36): ad lib use of own-brand VP (12 cartridge, 3 pod, 15 tank and 6 modular type) for 48 hours. Smoking (n=36): ad lib smoking of own-brand TC for 48 hours. Non-use (n=36): no use of tobacco or nicotine products for 48 hours.	HR: stat. sig. higher in smoking group compared with VP (p<0.01) and non-use group (p<0.01). Stat. sig. higher in VP group compared with non-use group (p<0.01). SBP: stat. sig. lower in non-use group compared with VP and smoking groups (both p<0.01). DBP: stat. sig. lower in non-use group compared with VP and smoking groups (both p<0.01).	Some concerns
Biondi-Zoccai et al., 2019, Italy (40)	Single use (A)	n=20 Smokers: self-reported healthy TC smokers with mean (SD) smoking time in years: 15 (12).	Cross-over conditions separated by 1 week. Vaping (n=20): 9 puffs of cartridge VP (Blu pro) with	SBP: stat. sig. increase within all groups after exposure. Stat. sig. higher in smoking versus HTP groups after	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Mean (SD) age: 35 (13), 70% females, mean (SD) BMI: 24 (5).	tobacco flavoured 16 mg/mL nicotine strength e-liquid. Smoking (n=20): smoking a TC (Marlboro Gold). Others (n=20): using a single Amber label heets with HTP (IQOS).	exposure (p=0.002). DBP: stat. sig. increase within all group after exposure. Stat. sig. higher in smoking versus HTP group after exposure (p=0.046).	
Chaumont et al., 2018, Belgium (37)	Single use (A)	n = 25 Smokers: self-reported healthy occasional smokers of <20 TC per week. Mean (SEM) age: 23 (0.4), 28% females, mean (SEM) BMI: 23 (0.4) kg/m2.	Cross-over conditions separated by 1 week. Vaping (n=25): 25 puffs with 30 s inter-puff interval and 4 s inhalation on a modular type VP (Alien 220 box mod, TFV8 baby beast tank, a dual Kanthal coil (V8 Baby-Q2 Core; 0.4Ω dual coils; Smoke) with 50%/50% PG/VG ratio e-liquid vaporised at 60 W, creating subohm vaping exposure. Sham vaping (n=25): the same puffing regime with turned off VP.	HR (n=10): NS diff. after exposure compared with baseline in both groups. Stat. sig. higher rate in VP group compared with non-use group 5 minutes (p=0.002) and 20 minutes (p=0.005) after exposure.	Low

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
Chaumont et al., 2018, Belgium (35)	Single use (A)	n = 25 Smokers: self-reported occasional smokers of <20 TC per week. Mean (SD) age: 23 (0.4), mean (SD) BMI: 23 (0.4).	Vaping, nicotine (n=25): 25 4-second puffs with 30 seconds inter-puff interval on a modular VP (Alien 220 box, 300 mAh 35 A variable voltage/wattage battery, 60 watts, 0.4 Ω dual coils) with 50%/50% PG/VG, 3 mg/mL nicotine strength vaping liquid. Vaping, non-nicotine (n=25): use of the same VP with 0 mg/mL nicotine strength vaping liquid. Sham vaping (n=25): same puffing on the VP which was turned off.	HR: stat. sig. increase within vaping and nnVP groups (both p<0.001) after exposure compared with baseline. NS diff. after exposure within sham vaping group. SBP: stat. sig. increase within vaping group after exposure compared with baseline (p<0.001). NS change after exposure within nnVP and sham vaping groups. DBP: stat. sig. increase within vaping (p<0.001) and nnVP (p<0.01) groups after exposure compared with baseline. NS change after exposure within sham vaping group. Arterial stiffness (augmentation index corrected for HR, AIx75): stat. sig. increase within vaping group after exposure (p=0.013).	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				NS change within nnVP (p>0.6) and sham vaping (p>0.3) groups after exposure. PWV (carotid-femoral): stat. sig. increase within vaping group after exposure (p<0.0001). NS change within nnVP (p>0.8) and sham vaping (p>0.4) groups after exposure.	
Chaumont et al., 2020, Belgium (36)	Single use (A)	n = 30 Vapers: self-reported former TC smokers, used VP for ≥1 year. Mean (SD) age: 38 (2), 100% males, mean (SD) BMI: 26 (1) kg/m2.	Cross-over conditions separated by at least 7 days: Vapers, nicotine (n=30): 10 puffs of modular VP (Alien 2020 box mod, 60 W, 0.4 Ω, 3000 mAh) with 50/50 PG/VG ration liquid with 1.5 mg/mL nicotine. Vapers, non-nicotine (n=30): same use of the same modular VP without nicotine. Non-use (n=30): sham	FU 20 minutes after exposure. HR: stat. sig. higher in VP nicotine group compared with nnVP and non-users' groups (p<0.001). NS diff. between nnVP and non-users' groups. SBP: stat. sig. higher in VP nicotine group compared with nnVP and non-users' groups (p<0.001). NS diff. between nnVP and non-users' groups.	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
			vaping of the same modular VP.	DBP: stat. sig. higher in VP nicotine group compared with nnVP and non-users' groups (p<0.001). NS diff. between nnVP and non-users' groups.	
Cobb et al., 2020, US (13)	5 days (A)	n = 22 Dual users: self-reported smoking ≥10 TC per day for ≥1 year and using a VP ≥3 times per week for ≥3 months. Expired air CO ≥10 ppm and urinary cotinine of 3/6 of NicAlert test strip. Mean (SD) age: 41.9 (13.2), 50% females, 50% white, 45.5% African American, 4.5% Middle Eastern, 4.5% Hispanic.	5-day cross-over conditions: Vaping (n=22): ad lib use of own-brand cartridge VP with 2.4%-4.8% nicotine strength and menthol (81.8%) or tobacco (18.2%) flavoured e-liquid. Dual use (n=22): ad lib use of own-brand VP and TC. Smoking (n=22): ad lib use of own-brand TC with menthol (81.8%) or non-menthol (18.2%) flavour. Non-use (n=22): no TC or VP use for the last cross-over condition.	HR (n=18): stat. sig. lower levels in VP group compared with dual and smoking groups (p<0.05). SBP (n=18): NS diff. between groups. DBP (n=18): NS diff. between groups	Some concerns
Cossio et al., 2020, US (42)	Single use (A)	n = 16 Non-users: self-reported tobacco naïve participants who have not used nicotine	Three cross-over conditions separated by ≥48 hours: Vaping (n=16): 18 4-second	FUs at 1 & 2 hours post-exposure. SBP: NS diff. within all	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		products in the last 6 months. Mean (SD) age: 24 (3), 43.8% females, mean (SD) BMI: 23.2 (2.8).	puffs every 20 seconds in 6 minutes on a cartridge type VP (White Cloud Cigarette) with menthol flavoured 5.4% nicotine strength e-liquid. Non-nicotine vaping (n=16): same use of the same VP with no nicotine e-liquid. Other (n=16): same use of a menthol cigarette-like pipe.	groups at all FUs. DBP: NS diff. within all groups at all FUs.	
Felicione et al., 2020, US (14)	Single use (A)	n = 43 Vapers (n=25): self-reported current VP use for > 3 months, use > 1 ml of liquid/ day with a nicotine concentration > 3 mg/ml, and smoking < 5 cigarettes/day. mean (SE) age: 24.4 (1.6), 4% females, 80% white) Smokers (n=18): self-reported smoking > 10 cigarettes/day for at least one year, < 5 lifetime	Vaping (n=43): 10 puffs every 30 seconds from a tank VP (eGo, 3.3 V, 1000 mAh battery and 1.5 Ω dual coil) with 70%/30% PG/VG unflavoured e-liquid with 18 mg/mL nicotine. After this, participants had two 5-minute ad lib VP use sessions separated by 30 minutes.	HR: NS diff. between vapers and smokers' groups. Stat. sig. increase within both groups after controlled and ad lib use sessions compared with baseline.	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		VP uses, and no VP use in the past month mean (SE) age: 30.4 (2.1), 22.2% females, 83.3% white).			
Franzen et al., 2018, Germany (15)	Single use (A)	n = 15 Smokers: self-reported, mean 2.9 pack years mean age 22.9, 67% female.	Vaping, nicotine (n=15): single use consisting of 10 4-second puffs every 30 seconds of cartridge VP (eGo-T CE4, 3.3 volts, 1.5 ohms and 7.26 watts), tobacco flavoured, 55% to 35% PG/VG ratio, 24 mg/mL nicotine. Vaping, non-nicotine (n=15): use of the same cartridge VP with 0 mg/mL nicotine e-liquid. Smoking (TC): a Phillip Morris TC.	HR: stat sig. increase in smoking and VP groups ($p<0.05$) Stat. sig. diff between TC (highest increase) and nicotine VP groups ($p<0.05$) NS diff. in nnVP group. SBP: NS diff. between cross-over conditions ($p=0.053$). NS diff after VP use ($p=0.088$), nnVP use ($p>0.05$) and smoking ($p=0.084$). DBP: Stat. sig. diff. between conditions ($p<0.05$). Stat. sig. decrease within 30 minutes after nnVP use ($p<0.01$), NS diff. within 15 minutes after smoking ($p=0.064$), NS diff. after VP use ($p>0.05$).	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				PWV: Stat. sig. diff. between conditions (p<0.01). Stat. sig. increase after 15 minutes after VP use (p<0.05) and smoking (p<0.01). NS diff. after nnVP use (p>0.05).	
Gonzalez et al., 2021, US (44)	Single use (A)	n = 15 Non-users of tobacco or nicotine products, confirmed with plasma cotinine Mean (SD) age: 21 (1), 40% females.	Vaping (n=15): 20 puffs with 30 seconds inter-puff interval of a pod VP (JUUL) with 30%/60% PG/VG, mango flavoured and 59 mg/mL nicotine strength. Vaping, no nicotine (n=15): same puffing regime of a tank VP (Smok Fit, variable voltage 10-16 V) with mango flavoured, 30%/70% PG/VG and 0 mg/mL e-liquid.	FU during and 10 minutes after exposure. HR: stat. sig. increase during and 10 minutes after exposure within VP group (p=0.002). NS diff. within nnVP group at both FUs (p=0.12). Stat. sig. diff. between VP and nnVP groups (Condition x Time, p=0.001). SBP: stat. sig. increase during and 10 minutes after exposure within VP group (p=0.021). NS diff. within nnVP group at both FUs (p=0.21). NS diff. between VP and nnVP groups (Condition x	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				Time, p=0.244). DBP: stat. sig. increase during and 10 minutes after exposure within VP group (p=0.001). NS diff. within nnVP group at both FUs (p=0.229). NS diff. between VP and nnVP groups (Condition x Time, p=0.051).	
Haptonstall et al., 2020, US (32)	Single use (A)	n = 136 Vapers (n=49): VP use for >1 year without smoking for >1 year, Co verified (CO<10ppm). Mean (SD) age: 27.4 (5.5), 26.5% females, 59.2% Caucasian, 26.5% Hispanic, 10.2% Hawaiian, 2.1% African American. Smokers (n=40): Smoking for >1 year, CO verified (CO>10ppm). Mean (SD) age: 27.1 (5.5), 35% females, 62.5% Caucasian, 20% Asian, 12.5% African American, 5% Hispanic.	Vaping (n=49, vapers): vaping a cartridge or pod VP (eGo-one, 1 Ω, or JUUL) for up to 60 puffs every 30 seconds with 1.2% nicotine strength strawberry flavour e-liquid (eGo-one) or 5% nicotine strength mint flavour salt (JUUL). Smoking (n=40, smokers): smoking own-brand TC in 7 minutes. Other (n=47, vapers): using nicotine inhaler with menthol flavour.	HR: stat. sig. increase within vapers' (p=0.0001) and non-users' (p=0.002) groups when using nicotine VP. Stat. sig. increase within smokers' group (p=0.00001) when using TC. NS change after using nnVP and nicotine inhaler. SBP: stat. sig. increase within vapers' (p=0.001) and non-users' (p=0.00001) groups when using nicotine VP. Stat. sig. increase within smokers' group (p=0.04)	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Non-users (n=47): non-smokers or former smokers for >1 year, CO verified (CO<10ppm). Mean (SD) age: 26.3 (5.2), 53.2% females, 55.3% Caucasian, 19.1% Asian, 10.6% Hispanic, 8.5% African American.		when using TC. NS change after using nnVP and nicotine inhaler. DBP: stat. sig. increase within vapers' (p=0.03) and non-users' (p=0.007) groups when using nicotine VP. Stat. sig. increase within smokers' group (p=0.03) when using TC. NS change after using nnVP and nicotine inhaler.	
Hiler et al., 2017, US (33)	Single use (A)	n = 64 Vapers (n=33): self-reported use of ≥ 1 mL e-liquid per day, use of ≥ 3 mg/mL nicotine e-liquid and use of a VP for ≥3 months, ≤ 5 cigarettes per day, CO ≤ 10ppm, Mean (SD) age: 30.3 (8.4), 18.2% females, 72.7% Caucasians. Smokers (n=31): ≥ 10 cigarettes per day, <5 VP use in lifetime, CO≥ 15ppm Mean (SD) age: 30.8 (9.9),	Four cross-over conditions separated by >48 hours: Vaping, 8 mg/mL (n=64): two vaping bouts 60 minutes apart consisting of 10 puffs every 30 seconds on a tank-type VP (eGo, 3.3 volt, 1000 mAh battery, 1.5 Ω dual coil) with 70%/30% PG/VG tobacco or menthol flavoured (chosen by participants) Vaping, 18 mg/mL (n=64): same procedure using	HR: stat. sig. increase within 8, 18 & 36 mg/mL nicotine groups after exposure (p<0.05). NS change within 0 mg/mL nicotine group after exposure (p>0.05).	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		41.9% females, 51.6% Caucasians).	18 mg/mL e-liquid. Vaping, 36 mg/mL (n=64): same procedure using 36 mg/mL e-liquid. Vaping, no nicotine (n=64): same procedure using 0 mg/mL e-liquid.		
Hiler et al., 2020, US (34)	Single use (A)	n = 32 Vapers/dual users: self-reported use of ≥1 mL e-liquid per day, use of ≥3 mg/mL nicotine e-liquid and use of a VP for ≥3 months. Smoking <5 CPD for ≤3 times per week. Mean (SD) age: 25.6 (7.1), 25% females, 59.4% Caucasian.	Four 3.5-hour cross-over sessions separated by ≥48 hours Vaping, 40.5 W, 0.5 Ω, 3 mg/mL nicotine (n=32): 10 puffs every 30 seconds and 60 minutes ad lib use separated by 60 minutes of a modular VP (Kangertech Subtank) with 30%/70% PG/VG, pear flavoured and 3 mg/mL nicotine strength e-liquid. Vaping, 40.5 W power, 0.5 Ω, 8 mg/mL nicotine (n=32). Vaping, 13.5 W power, 1.5 Ω, 3 mg/mL nicotine	HR: stat. sig. increase in all conditions after acute and ad lib vaping sessions. Stat. sig. higher increase after ad lib use in 3 mg/mL, 0.5 Ω. 40.5 W condition compared with 3 mg/mL, 1.5 Ω, 13.5 W condition ($p<0.05$).	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
			(n=32). Vaping, 13.5 W power, 1.5 Ω, 8 mg/mL nicotine (n=32).		
Ikonomidis et al., 2018, Greece (25)	Single use (A)	n = 70 Smokers attending hospital's smoking cessation unit. Mean (SD) age: 48 (5), 56% females.	Vaping (n=35): vaping for 7 minutes of a tank type VP (NOBACCO eGo Epsilon, 1100 mAh battery, 3.9 V) with 74.3%/20% PG/VG flavoured and 12 mg/mL nicotine strength e-liquid. Vaping, no nicotine (n=35): vaping for 7 minutes of the same VP with 0 mg/mL nicotine.	HR: NS diff. within all groups after acute exposure. SBP: NS diff. within all groups after acute exposure. DBP: NS diff. within all groups after acute exposure. Arterial stiffness (augmentation index corrected for HR, AIx75): NS diff. within all groups after acute exposure. PWV (carotid-femoral): NS diff. within all groups after acute exposure.	High
Ip et al., 2020, US (31)	Single use (A)	n = 145 Self-reported: Vapers (n=43)	Cross-over conditions separated by 4 weeks. Vaping (n=43): 60	HR: NS diff. in increased HR between VP and smokers' groups (p=0.10) and nnVP and smokers'	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Mean (SE) age: 28 (0.9), 37.2% females, mean (SE) BMI: 24.6 (0.6). Smokers (n=37) Mean (SE) age: 26.7 (0.9), 29.7% females, mean (SE) BMI: 24.3 (0.4). Non-users (n=65): Mean age NR, 44.6% females, mean (SE) BMI: 23.2 (0.4).	4-seconds puffs every 30 seconds of a cartridge VP (Greensmoke or eGo One, 1.0 Ω) with strawberry or tobacco flavoured e-liquid with 1.2% nicotine. Vaping, no nicotine (n=43): use of the same VP with 0 mg/mL nicotine. Smoking (n=37): smoking own-brand TC in 7 minutes.	groups (p=0.21). Stat. sig. higher increase in HR in VP compared with nnVP group (p=0.0005).	
Kerr et al., 2019, UK (9)	Single use (A)	n = 20 Smokers: self-reported smoking ≥1 TC per day. Mean (SD) age: 31.6 (10.5), all males, mean (SD) BMI: 25.7 (5).	Cross-over conditions separated by >24 hours. Vaping (n=20): 15 puffs on a tank type VP (1300mAh, 3.3 V battery voltage) with 66%/34% PG/VG ratio, 18 mg/mL nicotine strength and tobacco flavoured vaping liquid. Smoking (n=20): ad lib smoking of a TC.	HR: stat. sig. higher within VP (p<0.001) and smoking (p=0.001) groups after exposure compared with baseline. Stat. sig. higher increase after exposure in smoking compared with VP group (p<0.001). SBP: NS diff. after exposure compared with baseline within both groups. Stat. sig. higher increase after exposure in smoking compared with VP group (p=0.046).	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
				DBP: NS diff. within and between groups after exposure.	
Maloney et al., 2019, US (39)	Single use (A)	n = 24 Smokers: smoked ≥10 TC per day for >1 year, had expired CO ≥15 ppm and had used a VP <20 times in their lifetime. Mean (SD) age: 30.9 (9.5), 25% females, 25% non-Hispanic white, 45.8% non-Hispanic black.	Four cross-over conditions separated by ≥48 hours: Vaping (n=24): two 10 puffs sessions separated by 20 minutes of a tank VP (eGo, 3.3 V, 1000 mAh battery, 1.5 Ω dual coil) with 70%/30% PG/VG, tobacco or menthol flavoured and 36 mg/mL nicotine strength e-liquid. Vaping, no nicotine (n=24): two 10 puffs sessions separated by 20 minutes of the same VP with 0 mg/mL nicotine e-liquid. Smoking (n=24): 10 puffs of own-brand TC. Other (n=24): 10 puffs of a nicotine inhaler (10 mg nicotine, Pfizer).	HR: stat. sig. increase within VP, smoking and nicotine inhaler groups after first 10 puffs compared with baseline. Stat. sig. higher in smokers' group after first and second bouts of 10 puffs compared with VP and nnVP groups ($p<0.025$). Stat. sig. higher in VP group after second bout of 10 puffs compared with nicotine inhaler group ($p<0.025$).	Some concerns

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
Moheimani et al., 2017, US (50)	Single use (A)	n = 33 Self-reported non-users of VP or TC for ≥1 year. Mean (SD) age: 26.3 (0.9), 60.6% females, 45.5% white, 24.2% Asian, 15.1% black and 15.1% Hispanic.	Cross-over conditions separated by ≥4 weeks. Vaping (n=33): 60 3-second puffs with 30-seconds inter-puff intervals of a cartridge VP (Greensmoke or eGo One, 1.0 Ω) with tobacco (n=15) or strawberry (n=18) flavoured, 1.2% nicotine strength e-liquid. Vaping, no nicotine (n=33): vaping of the same VP with 0 mg/mL nicotine e-liquid. Sham vaping (n=33): vaping of the same VP without vaping e-liquid.	HR: stat. sig. higher increase in VP nicotine group after exposure compared with nnVP (p=0.05) and sham vaping (p=0.01) group. NS diff. increase in nnVP compared with sham vaping (p=0.54) group. SBP: NS diff. in change after exposure between groups (p=0.59). DBP: NS diff. in change after exposure between groups (p=0.23).	Some concerns
Rüther et al., 2018, Germany (41)	Single use (A)	n = 20 Vapers (n=9): vaping ≥3 months and had not smoked a TC for the past month. Mean (SD) age: 28.5 (8.9). Smokers (n=11): ≥5 TC per day for the past 3 years. Mean (SD) age: 26.2 (6.9).	Four cross-over conditions separated by 1 week: Vaping (n=9, vapers): 10 4-seconds puffs with 26 seconds inter-puff intervals of a cartridge (American heritage, Vype or Blu) or tank (Aspire/Joytech eGo C2, 650 mAh battery, 1.8 Ω) VP with strawberry/mint	FUs at 1-5 minutes after exposure. HR: stat. sig. increase within cartridge VP, tank VP and TC smoking groups at all FUs. Stat. sig. lower increase in cartridge VP group compared with tank VP (starting from 2 minute,	High

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
			flavoured, 18 mg/mL nicotine strength e-liquid. Smoking (n=11, smokers): 10 2-seconds puffs with 28 seconds inter-puff intervals of a Marlboro Red TC (0.8 mg nicotine).	p=0.011) and TC group (starting from 1 minute, p=0.001). Stat. sig. higher increase in TC group compared with tank VP group (starting from 2 minute, p=0.004)	
Spindle et al., 2018, US (38)	Single use (A)	n = 30 Vapers/dual users: healthy, smoking <5 TC per day, using ≥1ml of e-liquid per day and using a VP with ≥6mg/ml nicotine for ≥3 months. Mean (SD) age: 26.9 (7.1), 3.3% females, 70% Caucasian, 13.3% Asian, 6.7% African American, 10% of other ethnicity, mean (SD) CPD: 0.03 (0.2).	Vaping (n=30): two monitored sessions separated by 60 minutes using tank VP (eGo 3.3V battery with 1.5 Ω, dual-coil, 510 cartomizer, 7.3W) with 18 mg/mL nicotine of tobacco flavour for 10 puffs every 30 seconds. PG/VG ratios differed: 1) 100% PG 2) 55%/45% (NR) 3) 20%/80% 4) 2%/98%	HR: stat. sig. increase after sessions 1 and 2 within all conditions (p<0.05). NS diff. in change after exposure between all conditions.	Some concerns
Cross-sectional					
Boas et al., 2017, US (49)		n = 31 VP users (n=11: mean (SD) age: 29 (1.5), 2 females, 6 white, 1 African America, 1 Asian, 1 Hispanic.	Self-reported: Vapers (n=11): VP use most days for >1 year. Smokers (n=10): smoking	HR: NS diff. between groups (p=0.55). SBP: NS diff. between groups (p=0.47).	12/20

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Smokers (n=10): mean (SD) age: 27.1 (1.6), 2 females, 7 white, 1 Asian, 1 Hispanic. Non-users (n=10): mean (SD) age: 28 (1.6), 3 females, 6 white, 2 Asian, 1 Hispanic.	for >1 year. Non-users (n=10): no use of VP or TC or had stopped smoking >1 year.	DBP: NS diff. between groups (p=0.77).	
Demir et al., 2020, Turkey (48)		n = 76 Vapers (n=36): mean (SD) age: 41.7 (10.1), 22.2% females, mean (SD) BMI: 27.3 (5.8). Non-users (n=40): mean (SD) age: 39.1 (11.4), 25% females, mean (SD) BMI: 26 (3.4).	Vapers (n=36): self-reported VP use for ≥6 months. Non-users (n=40): self-reported no use of tobacco or nicotine products.	HR: stat. sig. higher in vapers compared with non-users' group (p<0.001). SBP: NS diff. between groups (p=0.534). DBP: NS diff. between groups (p=0.804).	9/20
Fetterman et al., 2020, US, (20)		n = 467 Vapers (n=36): mean (SD) age: 29 (6), 28% females. Dual users (n=52): mean (SD) age: 33 (7), 47% females.	VP users (n=36): current vaping ≥5 days a week, no current smoking for >3 months Dual users (n=52): current vaping and smoking ≥5 days a week, smoked >100 TC in their lifetime	Results were adjusted for age, sex, race and study site. HR: NS diff. between groups (p=0.1). SBP: Stat. sig. diff. between groups (p=0.007), with	10/20

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
		Smokers (n=285): mean (SD) age: 32 (7), 42% females. Non-users (n=94): mean (SD) age: 29 (6), 56% females.	Smokers (n=285): current smoking ≥ 5 days a week, no current vaping Non-users (n=94): no current use of nicotine products, smoked <100 TC in their lifetime, urinary cotinine <10 ng/mL	nonusers< VP users < smokers < DU. DBP: NS diff. between groups (p=0.14). PWV: NS diff. in carotid-femoral (p=0.12) and carotid-radial (p=0.2) PWV between groups.	
Kim et al., 2020, South Korea (45)		n = 7505 Self-reported: Vapers (n=62): mean age NR. Dual users (n=337): mean (SE) age: 36.7 (0.7). Smokers (n=4079): mean (SE) age: 43.6 (0.3). Non-users (n=3027): mean (SE) age: 39.8 (0.4)	Vapers (n=62): VP use for the past month and no smoking. Dual users (n=337): smoked >100 TC in lifetime, currently smoke and have used a VP in the past month. Smokers (n=4079): smoked >100 TC in lifetime, currently smoke and have not used a VP in the past month. Non-users (n=3027): smoked <100 TC in lifetime or never smoked and have not used a VP for the past	SBP: stat. sig. higher in smokers compared with never smokers' group (p<0.05). DBP: NS diff. between groups.	9/20

Author, publication year, country	Last follow-up (exposure length)	Participants' characteristics	Interventions/groupings	Study findings	Risk of bias
			month.		
Moon et al., 2020, South Korea (46)		n = 1208 100%men. VP users (n=63): Mean (SD) age: 37.1 (11.5). Smokers (n=715): Mean (SD) age: 42.3 (11.3). Non-users (n=430): Mean (SD) age: 38.4 (13.3).	Vapers (n=63): self-reported VP use at least once in the last month. Smokers (n=715): self-reported smoking >100 TC in their lifetime and currently smoking 'sometimes' or 'everyday'. Non-users (n=430): self-reported non-users of TC and VP and not former smokers.	Measures after >8 hours overnight abstinence. SBP: NS diff. between groups. DBP: NS diff. between groups.	11/20
Podzolkov et al., 2020, Russia (47)		n = 270 Self-reported: VP users (n=22). Smokers (n=51). Non-users (n=197). Mean (SD) age: 21.2 (2.3), 35.6% females.	Vapers (n=22): mean (95% CI) vaping duration: 4 (2-6) years; mean (95% CI) nicotine strength: 1 mg (0.8-1.6). Smokers (n=51): mean (95% CI) smoking duration: 3 (1.5-7) years; smoking pack-years: 0.9 (0.6-3.5); CPD: 6 (1.5-20).	SBP: NS diff. between groups. DBP: NS diff. between groups. Arterial stiffness (AI): stat. sig. higher in smokers and VP groups compared with non-users ($p<0.05$). NS diff. between VP and smokers' groups.	5/20

Notes: A – acute exposure; AI—augmentation index; CO – carbon monoxide; CVD—cardiovascular; DBP–diastolic blood pressure; FU – follow-up; HR–heart rate; L – long exposure; NS – non-significant; %O2–blood oxygen saturation; PWV – pulse wave velocity;

RCT – randomised controlled trial; S-M—short-medium exposure; SBP – systolic blood pressure; Stat. sig. diff. – statistically significant difference; TC – tobacco cigarette; VP – vaping product, nnVP – non-nicotine vaping product.

Cell studies

Study characteristics

Our search identified 2 studies investigating the effects of vaping product exposure on cardiovascular function (52, 53) (appendices: table 7). One study exposed human induced pluripotent stem cell (iPSC)-derived endothelial cells to participant's serum obtained after 10 minutes of vaping or smoking (52), while the other study used vaping product aerosol extract with 0 or 6 milligrams per millilitre (mg/mL) nicotine to expose human iPSC- derived cardiomyocyte culture and mouse atrial myocytes (HL-1 cells) (53).

Summary of findings

Lee and others (52) reported increased levels of reactive oxygen species (ROS) linked to endothelial dysfunction, as shown by altered tube formation, in human induced pluripotent stem cell (iPSC)-derived endothelial cells treated with serum previously obtained from vaping product users and tobacco cigarette smokers as compared with serum from non-smokers. The subsequent measurement of 62 inflammatory cytokines in the serum revealed increased levels of interleukin-6, sICAM-1, macrophage colony-stimulating factor, and monocyte chemoattractant protein 1 in vaping product users and smokers after vaping/smoking compared to baseline. Of note, cell viability and ROS levels varied considerably following treatment with e-liquids of different flavours with the cinnamon-flavoured vaping products being the most potent. Vaping-induced cytotoxicity was also assessed by Abouassali and others (53) who showed that vanillin and cinnamaldehyde flavoured vaping aerosol extracts were more toxic in HL-1 mouse atrial cardiomyocytes than fruit-flavoured extracts. In spontaneously beating human iPSC-derived cardiomyocytes, exposure to vaping aerosol extracts resulted in significant changes in the beating rate and prolonged the field potential duration, indicators of cardiac electrophysiological instability, with a greater effect observed in vanillin and cinnamaldehyde flavoured vaping extracts (53); this study is also discussed in chapter 6 on flavours in vaping products.

Animal studies

Study characteristics

There were 12 studies that assessed outcomes relevant to cardiovascular diseases (54-65). Another 4 studies that assessed multiple organ systems included cardiovascular-related outcomes (appendices: table 6) (10, 66-68).

Of the 16 studies, 13 used mice (10, 54-58, 60, 62-64, 66-68) especially C57BL/6 inbred strain, and 3 used rats (59, 61, 65). All animals were subjected to whole body inhalation exposure of vaping aerosol except for 2 studies that performed nose-only inhalation

exposure in mice (68) and anesthetised rats (61). Five studies compared the effects of vaping product aerosol exposure with that of tobacco cigarette smoke and air-control with the remaining studies investigating vaping product versus air exposures only. Vaping product liquid composition (nicotine, propylene glycol/vegetable glycerine (PG/VG) ratio, flavours), device characteristics as well as puffing regimes varied across studies, for example puff length varied from 2 seconds (61) to 10 seconds (57), puffs per day varied from 20 (63) to about 250 (59) and, where reported, overall study length from 2 weeks (57, 60) to 8 months (54) (appendices: table 6).

Summary of findings

In several studies that recorded cardiac sympathetic activity in mice, exposure to vaping product aerosol significantly increased both systolic and diastolic blood pressure (10, 63, 68) with one study showing nicotine- and time-dependent elevation in blood pressure with levels similar to that observed after exposure to tobacco cigarette smoke (63). While 4 studies revealed no effect on heart rate in mice exposed to vaping product aerosol and tobacco cigarette smoke (54, 56, 58, 63), 2 studies reported decreased heart rate following 2-week (57) and 6-month (68) vaping product exposure, however, (57) obtained the measurements under anaesthesia, which may not reflect values observed when conscious.

Most studies have focused upon effects on vascular and cardiac function in response to vaping product aerosol or tobacco cigarette smoke exposure. For example, a 6-month study in apolipoprotein E-deficient mice (ApoE-/-), a mouse model of atherosclerosis, demonstrated nicotine-related increase in arterial stiffness parameters (PWV; pulse propagation velocity) after vaping product aerosol exposure, with smaller impact relative to that of tobacco cigarette smoke exposure (62). The authors also found a significant effect of nicotine-containing vaping product aerosol, similar to that of tobacco cigarette smoke, on isovolumic relaxation time, a reliable index of diastolic function. These results were aligned with the earlier report of chronic (8 months) nicotine-containing vaping product exposure increasing PWV and impairing aortic endothelial function in C57BL6 mice, similarly to tobacco cigarette smoke exposure (54).

In one study, echocardiographic evaluation revealed greater left ventricular (LV) mass in vaping product exposed animals compared to both tobacco cigarette smoke and air-controls, while changes in fractional shortening (FS) and ejection fraction (EF), markers of LV function, were noted in the tobacco cigarette smoke group only (54). In contrast, Espinoza-Derout and others (56) and Hasan and others (58) found that 3 months of vaping product aerosol exposure with nicotine reduced both markers of LV function (FS and EF) and caused cardiomyocytes ultrastructural abnormalities indicative of cardiomyopathy in ApoE-/- mice on a western diet and a high-fat diet, in comparison with corresponding nicotine-free vaping product group or saline controls. However, these results were not replicated in the 6-month study by Szostak and others (62) using the same mouse model with a larger sample size per each group (n=8-12 vs. n=5). One more study evaluating the

effects of vaping product aerosol and tobacco cigarette smoke exposures on LV structure and function indicated significant increases in LV mass coupled with increases in vessel wall thicknesses (LV anterior and posterior wall thickness in diastole and systole) in both tobacco cigarette smoke and vaping product aerosol exposed C57BL/6 mice (6 to 14 months), leading to induction of cardiac hypertrophy (63). This was accompanied by increased adrenergic vasoconstriction and impairment of vascular endothelial relaxation. Although vaping product-induced changes were seen in the absence of nicotine, higher concentrations of nicotine exerted greater effect, similar to that of tobacco cigarette smoke exposures.

Vaping was also found to be associated with the induction of oxidative stress with increased production of ROS, therefore, contributing to the pathogenesis of cardiovascular disease. Indeed, the above studies reported that vaping product-exposed animals were found to have nicotine-related alterations in blood and heart tissue biomarkers linked to oxidative stress and endothelial dysfunction, including increased superoxide generation (63), elevated levels of malondialdehyde (MDA), indicating increased ROS generation along with mitochondrial DNA damage (56) and increased expression of 4-hydroxynonenal protein adducts (58). These observations are also in alignment with other data on increased oxidative stress markers in rats exposed to nicotine-containing vaping products compared with air-controls (59, 65). Additionally, Kuntic and others (10) showed that mice lacking the phagocytic NADPH oxidase (Nox-2) gene did not demonstrate oxidative stress and endothelial dysfunction following vaping product aerosol exposure, while the wild-type mice did, suggesting an important role of Nox-2 in mediating vaping product-induced oxidative stress, yet these findings were nicotine-independent. Endothelial dysfunction, as reflected by impaired FMD, has also been observed in rats after 10 puffs over 5 minutes of vaping product aerosol or tobacco cigarette smoke (61). Moreover, acute PG/VG exposure without nicotine or flavourings has been shown to affect endothelium-dependent relaxation as well as decrease white blood cells, and increase red blood cells and haemoglobin, which was believed to be a compensatory mechanism for supplementing blood oxygen levels (66). These effects were attributed to the presence of abundant saturated aldehydes, especially formaldehyde. In regular vaping, formaldehyde is generally only produced under extreme conditions which result in aversive ‘dry puff’ experiences and are avoided by people who vape (8).

Two studies have reported that vaping product aerosol exposure with nicotine caused hyperactive state of platelets, with enhanced aggregation, secretion and integrin and phosphatidylserine expression in C57BL/6 mice after one week (55) and 2 weeks (60). Importantly, both studies found altered haemostasis response as evidenced by shortened thrombosis occlusion and bleeding times; combined with enhanced platelet function these findings suggest an increased risk of thrombosis. Furthermore, vaping product aerosols with nicotine induced ventricular transcriptomic changes in genes associated with metabolism, circadian rhythm, and inflammation (56). Vaping product aerosol inhalation increased the levels of circulating inflammatory cytokines TNF-α and interleukin-6 (64),

pro-inflammatory proteins, including leukaemia inhibitory factor, epidermal growth factor and angiopoietin, as well as pro-fibrotic markers, such as collagen-3 (68), TGF-β and MMP-2 (59). Shi and others (57) reported that short-term exposure to vaping product aerosol increased heart tissue angiogenesis and endothelial cell markers (CD31 and CD34) but had no significant effect on cardiac fibrosis. In addition to the above biomarkers, Lee and others (67) found that a 12-week vaping product aerosol exposure with nicotine induced mutagenic DNA adducts (O6-methyldeoxyguanosines and γ-hydroxy-1, N2-propano-deoxyguanosines) in the heart tissues of FVB/N mice.

Summary of cell and animal studies

Cell studies indicated that vaping product aerosol increased damage to cells; effects varied across different flavours. Evidence was limited to 2 studies. Animal studies indicated that vaping product aerosol exposure increased blood pressure; some studies found a decrease in heart rate, although most found no effect. Animal studies also indicate an increase in markers of arterial stiffness linked to vaping product aerosol exposure which may be similar or smaller than increases caused by smoking. Left ventricular mass and vessel wall thickness were increased and left ventricular function reduced after vaping product aerosol exposure, potentially less than for smoking and there were inconsistencies in findings across studies. The above vaping-induced effects appear largely to be nicotine-dependent when directly compared between vaping product aerosol exposure with and without nicotine. Vaping exposure was associated with decreases in blood vessel health, as well as increases in markers of thrombosis risk, inflammation, oxidative stress, scarring, and cell health. Comparison between vaping product aerosol and tobacco cigarette smoke exposure provided mixed results on cardiovascular outcomes with several studies showing that vaping product-induced alterations were similar or less than those with tobacco cigarette smoke exposure.

There are several caveats concerning the validity of findings from animal studies for human outcomes. The variation in the animal studies included in this chapter indicate that there appears to be no consensus as to what exposure would mimic human exposure. As noted elsewhere (see chapter 9 on cancer), most animal studies used whole body exposure rather than nose-only exposure. This results in skin and oral exposure due to grooming during and after exposures, introducing other exposure routes not observed in humans who use vaping products. The conditions of experiments may induce other responses such as stress responses which are also associated with the outcomes studied here.

11.4 Conclusions

Our systematic review aimed to assess the effects of exposure to vaping on biomarkers associated with the risk of health conditions and to assess the effect of vaping on disease

outcomes in people with existing health conditions. For cardiovascular diseases, we did not identify any studies on people with existing cardiovascular conditions so we could not address the second aim of the review. We assessed both relative and absolute vaping risks associated with biomarkers of cardiovascular disease where the data were available (that is, between vapers and smokers, and between vapers and non-users), and where feasible, we included comparisons across different population groups.

We present our conclusions for biomarkers of exposure and biomarkers of potential harm cutting across several diseases in chapters 7 and 8. The studies we reviewed show that compared to smoking, using vaping products leads to a substantial reduction in biomarkers of toxicant exposure. However, the degree of any residual risk (from vaping but also prior smoking and other factors affecting cardiovascular health) remains unclear, mainly because of the lack of studies using appropriate comparators.

Looking at biomarkers of potential harm relevant to multiple diseases, studies of LDL cholesterol (sometimes described as ‘bad cholesterol’) showed no differences after acute and short-to-medium use of vaping products, smoking or non-use. Similar findings were observed for HDL cholesterol (‘good cholesterol’), except among large-scale samples of non-users where HDL levels were significantly higher than among vapers and smokers. The findings were more mixed for markers of oxidative stress 8-isoprostane and soluble Nox2-derived peptide. However, as these oxidative stress biomarkers are influenced by other factors, no strong conclusions could be made regarding their associations with vaping product use. For inflammation markers, heterogeneity of designs prevented us from making strong conclusions. The meta-analyses of cross-sectional studies suggested lower levels of the inflammation biomarkers (blood CRP and sICAM-1) among vapers than smokers, and similar levels between vapers and non-users, but these findings were not confirmed by other interventional studies that largely focused on acute and short-term exposure. For endothelial function biomarkers, a single RCT found that switching from smoking to vaping improved FMD after one month. Evidence from the other studies suggested a short-term deterioration in FMD after acute exposure to vaping product use. Evidence from the other endothelial function biomarkers and the 4 studies on platelet activation markers was also difficult to synthesise due to different designs, outcome measures and comparison groups.

We identified 41 studies that assessed biomarkers of potential harm specific to cardiovascular disease in humans. Consistent with studies in other chapters, the included studies used a range of different designs, had varying quality or risk of bias and used a range of different definitions of vaping and smoking. Studies with more than one time point mostly explored acute exposure to vaping or followed-up participants for short to medium term, so we were unable to summarise findings on longer-term vaping exposure. In line with our algorithm, we carried out meta-analyses wherever possible, but a lack of consistency in study designs, outcome reporting, group definitions and exposure periods resulted in data from few studies being meta-analysed.

Thirty-one studies assessed heart rate in humans (4 studies from the UK), and 9 of them could be included in meta-analyses. We were able to conduct 2 meta-analyses of findings comparing vaping and smoking (3 cross-over and 2 cross-sectional studies), 2 meta-analyses of findings comparing vaping and non-use (3 cross-over, 2 cross-sectional studies) and one meta-analysis of findings comparing vaping and non-nicotine vaping (4 cross-over studies). Acutely, vaping increased heart rate less than smoking. Heart rate after short exposure to vaping was similar to heart rate after no use of tobacco or nicotine products. There was no difference in heart rate after nicotine and non-nicotine vaping. Any differences may vary with devices, liquids and behaviours influencing the amount of nicotine delivered and this is further explored in chapter 5 on nicotine. Comparing longer-term changes in heart rate, people who vaped had lower heart rate than people who smoked when groups were mutually exclusive (that is, people who vaped did not also smoke). Compared with people who did not vape or smoke, heart rate among people who vaped was lower in a meta-analysis of cross-sectional studies but higher in another cross-sectional study. One longer-term study reported the same level of change in heart rate for smokers who started using nicotine or non-nicotine vaping products.

Thirty studies assessed blood pressure in humans (3 studies from the UK), 9 studies could be included in meta-analyses. We conducted 4 meta-analyses of findings comparing blood pressure when vaping and smoking (3 cross-over studies, 2 cross-sectional studies, meta-analysis repeated for systolic and diastolic blood pressure), 4 meta-analyses of findings comparing vaping and non-use (3 cross-over and 2 cross-sectional studies, again for both systolic and diastolic blood pressure) and 2 meta-analysis comparing nicotine and non-nicotine vaping (4 cross-over studies, again for both systolic and diastolic blood pressure). Meta-analyses comparing acute effects found no differences in blood pressure after vaping, smoking or doing neither with the exception of a small difference between vaping and non-use for diastolic blood pressure. Studies that could not be meta-analysed found mixed results. A meta-analysis comparing acute effects of nicotine and non-nicotine vaping found no difference as did most other studies that could not be meta-analysed but included non-nicotine vaping. Meta-analyses of cross-sectional studies where participants had had longer exposure to vaping (at least 3 months or one year) found that people who vaped (presumably mostly former smokers) had lower blood pressure than people who smoked. There was no difference between people who vaped and people who did not vape or smoke. Studies that could not be meta-analysed found mixed results regarding change in blood pressure.

Compared with cigarettes, vaping products produce no or little side-stream emissions, so we would expect effects on bystanders to be low. Only 2 small studies at serious risk of bias included secondhand exposure, so we could not draw any conclusions about what effects exposure to secondhand vapour has on heart rate or blood pressure.

Nine studies assessed peripheral resistance/arterial stiffness (PWV) in humans (one study from the UK). Results could not be meta-analysed. PWV may decrease (improve) after

smokers have switched to vaping for a sustained period, however, the longest follow-up reported was 4 months. PWV generally increased after acute exposure to vaping nicotine, but not after non-nicotine vaping, suggesting that any acute effects of vaping on PWV are due to nicotine.

Three studies (all at critical risk of bias, none from the UK) assessed acute effects on oxygen saturation in humans. Results could not be meta-analysed, and we could not draw conclusions based on the available evidence.

Evidence from cell studies was very limited, with only 2 studies identified in our review. Results showed that vaping product aerosol increased damage to cells and that effects varied across different flavours.

Sixteen studies in animals were included. In summary, animal studies indicated that exposure to vaping product aerosol increases blood pressure; some studies found a decrease in heart rate, although most found no effect. Animal studies also showed an increase in biomarkers of arterial stiffness linked to exposure to vaping products. This may be similar to or smaller than increases caused by smoking. Left ventricular mass and vessel wall thickness were increased and left ventricular function reduced after vaping product aerosol exposure. These effects were potentially less than for exposure to cigarette smoke, and there were inconsistencies in findings across studies. These vaping product-induced effects appeared largely to be nicotine-dependent. Exposure to vaping product aerosol was associated with decreases in animals' blood vessel health, as well as increases in markers of thrombosis risk, inflammation, oxidative stress, scarring, and cell health although it is inconclusive as to which constituents of the aerosol play key roles in the observed effects.

As previously mentioned, it is challenging to directly translate the findings from pre-clinical studies using human or animal cells or rodent models to any cardiovascular risks arising from vaping in humans. These pre-clinical studies commonly employ acute exposures sometimes over concentrated periods, and it is unclear whether the mechanisms or pathways to risk identified would be replicated in people who vape.

The evidence does not allow us to distinguish pathways to cardiovascular disease. One potential pathway is through nicotine, and the biomarkers of exposure and pharmacokinetic studies indicate that people who vape can achieve nicotine levels similar to people who smoke. The animal studies suggested that nicotine did play a role in some of the changes observed in cardiovascular biomarkers, specifically blood pressure, arterial stiffness, left ventricular mass and function. Some studies included in this chapter assessed cardiovascular biomarkers in humans through non-nicotine vaping as well as nicotine vaping, which could help clarify the putative role of nicotine in any cardiovascular risks of vaping for humans. However, the heterogeneity of studies limits conclusions. Meta-analyses of cross-over studies from vaping nicotine and non-nicotine products for heart rate and blood pressure found no differences. Studies that we could not meta-

analyse did not consistently find this. The findings were more consistent in relation to PWV effects where nicotine did appear to be implicated at least in acute studies.

Conclusions from the NASEM report are generally supported by this present review. As in 2018, to date there is still no available evidence regarding whether vaping is associated with clinical cardiovascular outcomes (coronary heart disease, stroke, and peripheral arterial disease) and subclinical atherosclerosis (carotid intima-media thickness and coronary artery calcification). The NASEM report found substantial evidence that heart rate increased shortly after nicotine intake from vaping which was also seen in this present review (whereas evidence was inconsistent for non-nicotine vaping). NASEM found moderate evidence that diastolic blood pressure increases shortly after nicotine intake from vaping and limited evidence that vaping is associated with a short-term increase in systolic blood pressure. Based on the still limited and mixed evidence, we conclude that there may be reductions in blood pressure after people who smoke switch to vaping and little difference between people who vape and people who do not vape or smoke. The NASEM report also concluded that there was insufficient evidence that vaping was associated with long-term changes in heart rate, blood pressure, and cardiac geometry and function. In our review, evidence from animal studies suggests that there may be some long-term changes, but we found no evidence from human studies. And, as discussed, the validity of animal studies for human outcomes has limitations.

Similarly, conclusions by COT are generally supported by this present review. COT concluded that exposure to nicotine from vaping was unlikely to be higher than from smoking – this is confirmed by studies included in this present review finding no significant difference between people who vaped or smoked at least weekly. COT also concluded that vaping was associated with some emissions into ambient air, including nicotine, so that pharmacological effects from exposure to nicotine in ambient air may occur in some individuals. In the present review, only 2 small studies at serious risk of bias assessed short-term second-hand exposure to nicotine vaping allowing no clear conclusions.

Overall, the extent to which vaping presents a risk for cardiovascular health remains uncertain, but based on the toxicant profile in vaping products and aerosols is expected to be much less than that of cigarette smoking.

11.5 Implications

Our quality assessments revealed most studies had some methodological concerns, and these should be addressed in future research as they limit interpretations of our findings. More research is needed, particularly in the UK, where we identified a dearth of studies.

Most studies exposed participants to brief sessions of vaping (27 out of 41 included studies were cross-over or acute exposure studies). While relevant to address questions

around immediate effects of vaping, this study design is not able to answer questions about effects on cardiovascular health outcomes most relevant to public health.

Studies that compare rates of cardiovascular diseases between non-users, users of tobacco and users of nicotine vaping products are needed (for example coronary heart disease, peripheral arterial disease, stroke).

Studies should include longer-term follow-ups and more informative measurements. Studies measuring heart rate or blood pressure should endeavour to include 24-hour ambulatory blood pressure and heart rate. This would improve the validity of the measurement rather than rely solely on measurements in single or short sessions. Researchers should consider including heart rate variability (a higher variability can indicate better health) as an outcome measure, for example in people who switch from smoking to vaping. Evidence is also needed on the extent of longer-term changes in other outcomes such as pulse wave velocity. Alongside longer follow-ups, inclusion of long-term exclusive vapers may also help address this.

Historical tobacco use can greatly affect many of the biomarkers used to determine exposure to potentially harmful constituents from vaping. As most vapers are previous long-term smokers (see chapter 4 on vaping among adults), definitions for vaping should preclude concurrent smoking and a minimum duration of exclusive vaping should be defined. Studies are needed that compare long-term former smokers who do and do not vape as well as studies comparing former smokers who vape with people who vape who have never smoked.

In addition, compliance with study allocation and definitions of groups should be verified and reported in all studies, for example the level of CO exhaled by people categorised as not smoking and the level of nicotine in people categorised as vaping or not using any nicotine products.

The existing evidence does not provide insights into the effects of vaping on cardiovascular health in people of different gender, age or ethnicity and future research should pay attention to groups with different cardiovascular risk profiles.

Studies are in particular needed that assess the effects of vaping on people with pre-existing cardiovascular conditions, both in comparison with no use of nicotine or tobacco and in comparison with smoking.

Cardiovascular health and disease are affected by a wide range of genetic predispositions, behavioural risk factors and environmental exposures; further research is needed to clarify any unique contributions from vaping while accounting for other factors.

Vaping products vary and any effects on cardiovascular health are likely to differ with device types, nicotine concentration, liquid composition and user behaviours. As one

example, most studies in the US used nicotine concentrations above the legal threshold in the UK and EU, but we were unable to run meta-analyses comparing effects of nicotine concentration on outcomes.

For policy makers and practitioners, findings from our review for this chapter suggest that developing and implementing policies and interventions that support smokers to completely switch from smoking to vaping will reduce exposure to toxicants and carcinogens with relevant outcomes for cardiovascular health.

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12 Other health outcomes

12.1 Introduction

The previous chapters focused on the 3 main causes of smoking-related premature morbidity and mortality, namely cancers, cardiovascular diseases and respiratory diseases. This chapter aims to give an overview of studies identified in our systematic review which present evidence on associations between vaping and health conditions outside the scope of the previous chapters. Previous reports (1-3) have provided evidence on vaping among people with mental health problems, reproductive health effects, developmental effects and oral health but have not covered other outcomes.

12.2 Summaries of previous reports

Reproductive health and developmental effects

The report commissioned by Public Health England (PHE) and published in 2020 (1), included a chapter on vaping in pregnancy. A systematic review was conducted to address several research questions, including what health outcomes had been reported in studies of vaping in pregnancy and what findings had been reported for these outcomes. The review identified 2 publications which reported on health outcomes related to vaping. Both publications reported on a single small study (for example, only 6 exclusive vapers were included). The main findings of the review overall were that there was a lack of evidence on the prevalence of vaping in pregnancy in England, the effects of vaping on smoking during pregnancy and following childbirth, and on the effects of vaping on maternal health or pregnancy outcomes. Findings also included that as in other populations, pregnant women who vape were likely to do so to stop smoking, that vaping in pregnancy was very rare among those who have not smoked, that pregnant smokers and health professionals were unsure about the relative risks of vaping for mother and baby, and that clinical practice on vaping in pregnancy varied (1).

A peer-reviewed paper was published based on an updated version of the systematic review which included a further study on health-related outcomes (4). It reported that of 3 studies with health-related outcomes, 2 were underpowered and one reported similar birthweight for babies born to non-smokers and women who vaped, which was higher than the birthweight of babies born to women who smoked. It concluded that there were insufficient data to draw conclusions about prevalence, patterns, and effects of vaping in pregnancy on smoking cessation and that the limited literature suggested that vaping in pregnancy had little or no effect on birthweight.

The 2018 National Academies of Sciences, Engineering, and Medicine (NASEM) report (2) assessed the evidence for developmental and reproductive effects. There were no studies in humans and a very small number of animal (3) and cell (one) studies. The authors summarised that given the lack of direct empirical evidence of e-cigarettes' effects on the mother or foetus, from either human or animal studies, little could be said regarding an integrated evaluation. Their conclusions were that there was no available evidence whether or not vaping affects pregnancy outcomes and that there was insufficient evidence whether or not maternal vaping affects foetal development (2).

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) (3) summarised that there was a small number of studies that had investigated potential developmental toxicity of vaping product aerosols in animal models which supported the conclusion of effects of nicotine on development of the neurological and respiratory systems. However, it was not possible to quantify relative risks for developmental toxicity, nor to conclude specifically that nicotine was the sole contributing factor in these studies (3).

Dental and oral health

The 2018 NASEM report (2) reviewed findings on the associations between vaping and oral diseases. The review included 4 studies in humans and 5 in vitro studies. The synthesis of the evidence was that there were no epidemiological studies examining the associations between vaping and incidence or progression of periodontal disease. Based on the available evidence, the authors concluded that there was limited evidence suggesting that switching to vaping products would improve periodontal disease in smokers. They also concluded that there was limited evidence suggesting that nicotine and non-nicotine–containing vaping product aerosol could adversely affect cell viability and cause cell damage in oral tissue in non-smokers (2).

Oral or dental health effects have not been covered in any of the reports commissioned by PHE or the COT statement. In a 2020 systematic review of the oral health impact of vaping, the authors concluded that "although switching to e-cigarettes may mitigate oral symptomatology for conventional smokers, findings from this review suggest that a wide range of oral health sequelae may be associated with e-cigarette use" (5). More recently, Holliday and others reviewed the evidence on possible oral health effects of vaping products using basic science studies that evaluated cell lines and tissue cultures (in vitro studies), microbiological evidence from basic science and clinical research, evidence from clinical studies evaluating oral health and smoking cessation (in dental settings), and evidence from epidemiological studies (6). They concluded that in vitro studies had reported a range of cellular effects, but these were much less pronounced than those resulting from exposure to tobacco smoke. For microbiological studies, they concluded that vaping product users may have a different composition of bacteria in their mouth, with some indication that vaping product users might be at higher risk of disease than people

not smoking or vaping. Evidence of oral health effects from clinical studies was limited, and most studies had been small and cross-sectional. Epidemiological studies highlighted concerns over oral dryness, irritation, and gingival diseases. Overall, Holliday and others concluded that studies revealed potential oral health harms, underscoring the importance of efforts to reduce use in non-smokers. However, they also highlighted that in smokers who are using e-cigarettes as an aid to help them quit, the benefits of quitting tobacco smoking may outweigh any negative oral health impacts of e-cigarette use, particularly in the short term. Both Yang and others (5) and Holliday and others (6) called for well-conducted research. This will help understand the clinical significance of some of the biological changes observed and provide evidence on oral health outcomes of vaping product use.

12.3 Findings from the present systematic review

We included 28 studies in humans, 31 in animals and one in cells in this summary. The risk of bias was assessed using different tools depending on the study design. Full results of the risk assessments are in the Appendix, the summary scores are included in the tables in this chapter.

Studies in humans

The largest group of studies reporting on other health outcomes covered dental health outcomes. Our review initially identified 32 studies in this area; of those, 27 had been conducted in humans, 3 in cells and 2 in bovine enamel samples. Here, we report only on the 15 human studies that were not included by Holliday and others or Yang and others in their reviews (5, 6). Biomarkers of potential harm cutting across multiple diseases (mostly on inflammation) from dental or oral health studies are presented in chapter 7 together with other studies reporting on these outcomes (7-9).

Of the 15 dental health studies, 5 were longitudinal and 10 cross-sectional. Ten had been conducted in Saudi Arabia, one in the US (10), one in Italy (11), one in Malaysia (12) and for one, no country could be determined from the publication (7). The longitudinal studies included between 60 (13) and 18,259 (10) participants and followed them up for 3 or 6 months or 3 years (10). Two included only male participants (13, 14), one 87% men (15) and the final one included 76% men (12), with women included almost exclusively in the non-smoker group. The cross-sectional studies included between 90 (11) and 160 participants (16); 8 of them included only male participants, one (7) included 92% men and one (11) 70% men. Mean ages were generally in the 30s and 40s. Risk of bias was serious or critical for all longitudinal studies, the cross-sectional studies scored between 6 and 11 out of 20 (table 1 and appendices). Commonly reported outcomes were around plaque, gum health and bone loss. Smoking and vaping status were self-reported without biochemical verification and little to no information on vaping behaviour. Results are

presented in table 1. Briefly, smokers in general appeared to have worse outcomes than vapers and vapers often, but not always, had worse outcomes than non-users.

Among 13 studies in humans reporting on other health outcomes, 11 were cross-sectional, presenting whether or not there were associations between vaping and a range of health outcomes and 2 studies measured effects of acute exposure. Again, smoking and vaping status were self-reported without biochemical verification and little to no information on vaping behaviour. Risk of bias assessment was 8 to 17 out of 20 for cross-sectional studies and some concerns/moderate risk for the acute exposure studies (see tables and appendices for details). As cross-sectional studies, they do not allow conclusions about causal effects, any associations may also be caused by other characteristics, behaviours or exposures; for example, studies often did not report if vaping product users had previously smoked, making it difficult to disentangle effects from vaping and smoking. Additionally, health outcomes measured are affected by a wide range of other exposures, behaviours and predispositions which often were not or only partially accounted for.

Four cross-sectional studies and 2 acute exposure studies (table 2) reported on ocular health (17-20). This group of studies was the only that reported some information on liquids used. In the cross-sectional studies, vaping product users had significantly worse measures on the included outcomes than non-users, while the acute exposure studies reported no differences between smoking, vaping or non-use.

Three cross-sectional studies (table 3) reported on outcomes relevant for sexual or reproductive health (21-23). One of them found statistically significantly lower sperm count among men who vaped compared with those who did not vape or smoke, the other reported no statistically significant difference for fecundity ratio. The third study (22) reported that HPV-16 infection rates were higher among people who vaped compared with non-users; this study is included in the cancer chapter (chapter 6).

Two cross-sectional studies (table 4) reported on pre-diabetes or insulin resistance (24, 25), one finding a statistically significant association (higher rates of self-reported prediabetes in people who vaped compared with people who did not vape), the other not finding statistically significant associations (with insulin resistance).

Two cross-sectional studies (table 5) reported on outcomes related to self-reported asthma or allergies (26, 27). They reported an increased risk, particularly for people who (also) smoked.

Single studies reported on other health outcomes and any associations with vaping (table 6). One cross-sectional study reported on a number of self-reported health problems (28), some which the authors combined as tobacco related diseases (diabetes, asthma, cerebrovascular diseases, chronic obstructive pulmonary disease (COPD), cancer). They found no statistically significant associations between the tobacco related diseases and either ever or current e-cigarette use, for either men or women. One cross-sectional study

reported that sleep quality of dual users was statistically significantly worse than that of non-users, vapers or smokers (29). One cross-sectional study reporting on kidney disease concluded that occasional vaping was not a statistically significant additional risk factor (30).

Table 1. Studies in humans on periodontal and related health outcomes and any associations with vaping, by study design

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
Akram et al., 2021, Saudi Arabia, Longitudinal, (13)	n = 60, 100% male; mean age 35.74 (SD 14.52, range 29.3-58.6) Presenting at least 2 non-adjacent healthy sites and 2 periodontitis sites Vapers n=30, smokers n=30	Vapers (n=30): self-reported use ≥2 years Smokers (n=30): smoking ≥2 years	BoP: significant increase in vapers compared to smokers ($p<0.05$) PD: significant increase in smokers at 6 months compared to baseline; higher and more rapid increase in smokers compared to vapers ($p<0.01$) CAL: significant increase for both groups at 6 months only when compared to baseline ($p<0.01$); smokers significantly higher than vapers ($p<0.01$) MBL: both groups presented differences at 6 months ($p<0.01$); smokers significantly higher increase compared to vapers at 6 months ($p<0.001$)	Critical
Al-Hamoudi et al., 2020, Saudi Arabia, Longitudinal (15)	n = 71 Patients diagnosed with CP Vapers (n=36): mean age 47.7 (SD 5.8), men:women 32:4 Non-smokers (n	Vapers (n=36): self-reported daily use for past 12 months and no tobacco product Non-smokers (n=35): never used any tobacco product (including vaping products)	**Baseline** PI, PD, CAL, marginal BL: no statistically significant differences between vapers and non-smokers; GI: significantly higher in non-smokers vs vapers ($p<0.05$) **3-month FU** PI, GI, PD, CAL, marginal BL: vapers no statistically significant difference compared to baseline ($p>0.05$); PI, GI, PD significantly lower in non-smokers ($p < 0.05$); CAL, MBL: no statistically significant difference between vapers and non-smokers.	Serious

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
	= 35): male:female 30:5; mean age 47.7 (SD 5.8)			
ALHarthi et al., 2019, Saudi Arabia, Longitudinal (14)	n = 89, 100% male Presenting at least 30% BoP Vapers (n=28): mean age 32.5 (SD 4.8) Smokers (n=30): mean age 36.4 (SD 2.8) Non-smokers (n=31): mean age 32.6 (SD 3.5)	Vapers (n=28): self-reported daily exclusive vaping product use for previous 12 months, no previous history of tobacco usage Smokers (n=30): self-reported ≥5 cigarettes daily for previous 12 months Non-smokers (n=31): self-reported never use of any tobacco product	Within smokers: PI, PD and no of sites with PD≥4mm in smokers: significantly higher at 3 months f/u compared with baseline. No significant difference at 6 months compared to baseline and 3 months. BoP no sig difference at all time points. Within vapers: PI, PD significantly higher at baseline compared with 3 and 6 month f/u in vapers. BoP no significant difference at all time points. No of sites with PD≥4mm were higher at baseline compared with 0 sites at 3 and 6 month f/u. Within non-smokers PI, BoP and PD significantly higher at baseline compared with 3 and 6 month f/u. No of sites with PD≥4mm were higher at baseline compared with 0 sites at 3 and 6 month f/u. Smokers vs vapers: PI, BoP, PD and no of sites with PD≥4mm no significant difference at baseline. At 3 and 6 month f/u, PI and PD were significantly higher in smokers vs vapers ($p<0.05$) and no of sites with PD≥4mm were higher in smokers compared with – sites in vapers. BoP no statistically significant difference at all time points. Vapers vs non-smokers: PI, PD and no of sites with PD≥4mm	Serious

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
			no significant difference at baseline but BoP was significantly higher in non-smokers compared with vapers (p<0.01). PI, BoP and PD no significant difference at 3 and 6 month follow up. No pockets with PD≥4mm at 3 and 6 month FU in either group. MT: no difference across groups CAL: not presented in any of the groups	
Atuegwu et al., 2019, US, Longitudinal (24)	n = 18,259 Participants who reported 'no gum diseases' at baseline and completed waves 1, 2 & 3 of the PATH survey. 46.3% male Age groups: Vapers, longitudinal: 18-24: 23.8%, 25-34: 30.8%, 35-44: 15.9%, 45-54: 14.4%, 55 and older: 15.1% Vapers, non-longitudinal: 18-	Vapers, longitudinal (n=329): self-reported regularly using vaping products in all three survey waves; 38.4% were longitudinal tobacco cigarette smokers, and a further 38.6% former cigarette smokers. Vapers, non-longitudinal (n=8,298): self-reported ever nicotine vaping product users that did not use every day or some days in	Longitudinal vapers had increased odds of being diagnosed with gum disease (OR 1.07, 95%CI:1.12-2.76), bone loss around the teeth (OR 1.67, 95%CI:1.06-2.63), and any periodontal disease (OR 1.58, 95%CI: 1.06-2.34) compared to never vapers after adjusting for longitudinal cigarette use and other confounders.	Critical

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
	24: 30.8%, 25-34: 29.0%, 35-44: 16.6, 45-54: 12.4%, 55 and older: 11.1% Never vapers: 18-24: 9.6%, 25-34: 15.7%, 35-44: 17.4%, 45-54: 19.3%, 55 and older: 38.0%	the three survey waves. 40.1% and 14.5% were longitudinal smokers and former smokers respectively. Never vaper (n=9,632): 4.3% and 20.5% were longitudinal smoker and former smokers respectively.		
Ghazali et al., 2019, Malaysia, Longitudinal (12)	n = 135 Vapers (n=45): 4.4% (n=2) female; mean age 22.92 (SD 2.91); 95.6% (n=43) Smokers (n=45): 0.7% (n=1) female; mean age 30.28 (SD 8.31) Non-smokers	Vapers (n=45): NR Smokers (n=45): NR Non-smokers (n=45): NR	DMFT score (sum of number of decayed, missing due to caries, and filled teeth in permanent teeth): no significant difference of mean between groups at baseline and FU; significant difference of median score within groups between baseline and FU (vapers $p < 0.001$; smokers $p = 0.005$; non-smokers $p = 0.023$).	Critical

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
	(n=45): 64.4% (n=29) female; mean age 29.78 (SD 9.74)			
Al-Aali et al., 2018, Saudi Arabia, Cross-sectional (8)	n = 92, 100% male Vapers (n = 47): mean age 35.8 (SD 6.2) Non-smokers (n =45): mean age 42.6 (SD 2.7)	Vapers (n = 47): self-reported vaping product use for past 12 months. Non-smokers (n =45): self-reported never use of any tobacco product	Peri-implant BoP: significantly higher among in non-smokers compared to vapers ($p<0.01$). PD ≥ 4mm: significantly higher in vapers compared to non-smokers ($p<0.05$). PI: no statistically significant difference between the groups. BL: significantly higher in vapers compared to non-smokers ($p<0.05$)	11/20
Alqahtani et al., 2019, Saudi Arabia, Cross-sectional (9)	n = 102, 100% male Patients partially edentulous and rehabilitated with dental implants Vapers (n=34): mean age 33.5 (SD 0.7) Smokers (n=35): mean age 36.3 (SD 1.2)	Vapers (n=34): self-reported daily vaping product use for past 12 months Smokers (n=35): self-reported daily cigarette smoking for at least 12 months. Waterpipe users (n=33): self-reported daily use for at least 12 months	PI, PD: significantly higher among vapers, smokers and waterpipe users compared with non-smokers ($p<0.05$). Peri-implant BoP: significantly higher in non-smokers compared to vapers, smokers and waterpipe users ($p<0.05$)	10/20

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
	Waterpipe users (n=33): 34.1 (SD 1.4) Non-smokers (n=35): 32.2 (SD 0.6)	Non-smokers (n=35): self-reported never use of any tobacco products		
AlQahtani et al., 2018, Saudi Arabia, Cross-sectional (16)	n = 160, 100% male Vapers (n=40): mean age 35.6 (SD 7.1) Smokers (n=40): mean age 45.8 (SD 6.8) Waterpipe users (n=40): mean age 43.5 (SD 4.9) Non-smokers (n=40): mean age 42.6 (SD 2.7)	Vapers (n=40), smokers (n=40), waterpipe users (n=40): self-reported >10 cigarettes daily for at least 5 years Non-smokers (n=40): self-reported never use of any tobacco products	PI: significantly higher among vapers, smokers and waterpipe users compared to non-smokers ($p<0.05$) BoP: significantly higher in non-smokers compared to vapers, smokers and waterpipe users ($p<0.01$) PD ≥ 4mm: significantly higher in smokers and waterpipe users compared to vapers ($p<0.05$) Radiographic BL: significantly higher in vapers ($p<0.05$), smokers ($p<0.01$) and waterpipe users ($p<0.05$) compared to non-smokers; statistically significant in smokers and waterpipe users compared with vapers ($p<0.05$)	10/20
ArRejaie, et al., 2019, Saudi	n = 95, 100% male	Vapers (n=31): self-reported vaping product use in	PI: significantly higher in vapers and smokers compared to non-smokers ($p<0.01$); significantly higher in smokers compared to vapers ($p<0.01$)	10/20

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
Arabia, Cross-sectional (62)	Vapers (n=31): 35.8 (SD 6.2) Smokers (n=31): 40.4 (SD 3.5) Non-smokers (n=32): 42.6 (SD 2.7)	previous 12 months Smokers (n=31): self-reported cigarette smoking in previous 12 months Non-smokers (n=32): self-reported never use of any tobacco products	BoP: significantly higher in non-smokers compared to vapers and smokers (p<0.01) PD ≥ 4mm: significantly higher in vapers and smokers compared with non-smokers (p<0.01) MBL: significantly higher in vapers and smokers compared to non-smokers; significantly higher in smokers compared to vapers (p<0.01)	
Bardellini et al., 2018, Italy, Cross-sectional (11)	n = 90 Vaper (n=45): male:female 41:4; mean age 47 (SD 10) Former smokers (n=45): male: female 22:23; mean age 47 (SD 11)	Vapers (n=45): self-reported current vaping product use for at least 6 months Former smokers (n=45): self-reported daily or almost daily smokers (≥100 cigarettes) and quit smoking at least 6 months to a maximum of 24 months prior to the study.	Oral mucosal lesions: higher prevalence among vapers (65.4% vs 34.6%) but not statistically significant. Increased prevalence of nicotine stomatitis (p<0.04), hairy tongue (p<0.02) and hyperplastic candidiasis (p<0.04) in vapers	8/10
Binshabaib, et al., 2019, not reported,	n= 135 Vapers (n=44):	Vapers (n=44): self-reported daily vaping product use	PI, PD and CAL: significantly higher in smokers compared to non-smokers (p<0.05); no significant differences between vapers and non-smokers	10/20

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
Cross-sectional (7)	male:female 42:2; mean age 36.5 (SD 1.7) Smokers (n=46): male:female 43:3; mean age 44.2 (SD 3.5) Non-smokers (n=45): male:female 39:6; mean age 40.6 (SD 3.3)	Smokers (n=46): self-reported ≥5 cigarettes daily over 12 months Non-smokers (n=45): self-reported never use of any tobacco products People who used more than one product were excluded.	BoP: significantly more often manifested in non-smokers compared to vapers and smokers ($p<0.05$). MBL: significantly higher in smokers compared to non-smokers ($p<0.01$); no significant differences between vapers and non-smokers MT: no significant differences between vapers and non-smokers	
Ibraheem et al., 2020, Saudi Arabia, Cross-sectional (63)	n = 120, 100% male Vapers (n=30): mean age 45.6 (SD 3.6) Smokers (n=30): mean age 46.5 (SD 5.3) Waterpipe users (n=30): mean age 45.5 (SD 4.4)	Vapers (n=30): self-reported daily vaping product use for previous 12 months Smokers (n=30): self-reported ≥5 cigarettes daily for previous 12 months Waterpipe users (n=30): self-reported exclusive daily use for previous 12	PI, PD, CAL and marginal BL higher in vapers, smokers and waterpipe users compared to non-smokers ($p<0.01$) BoP: no statistically significant differences across groups	9/20

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
	Non-smokers (n=30): mean age 43.8 (SD 1.7)	months Non-smokers (n=30): self-reported never use of any tobacco products People who used more than one product were excluded.		
Javed et al., 2017, Saudi Arabia, Cross-sectional (64)	n = 94, 100% male Vapers (n=31): mean age 37.6 (SD 2.1) Smokers (n=33): mean age 41.3 (SD 2.8) Non-smokers (n=30): mean age 40.7 (SD 1.6)	Vapers (n=31): self-reported exclusive daily vaping product use for at least 12 months Smokers (n=33): self-reported ≥5 cigarettes daily for at least 12 months Non-smokers (n=30): self-reported never use of any tobacco products People who smoked and vaped were	DP, PD ≥4mm: significantly higher in smokers compared to vapers and non-smokers (p<0.01) BoP: significantly higher in non-smokers compared to vapers and smokers (p<0.01) MT, CAL, MBL: no statistically significant difference Self-rated gingival pain: more often reported by smokers than vapers and non-smokers (p<0.01); no difference between vapers and non-smokers Self-rated gingival swelling: more often reported by smokers than vapers and non-smokers (p<0.01); no difference between vapers and non-smokers Self-rated gingival bleeding: more often reported by non-smokers than vapers and smokers (p<0.01); no difference	6/20

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
		excluded	between vapers and smokers.	
Mokeem,et al., 2018, Saudi Arabia, Cross-sectional (65)	n = 154, 100% male Vapers (n=37): mean age 28.3 (SD 3.5) Smokers (n=39): mean age 42.4 (SD 5.6) Waterpipe users (n=40): mean age 44.7 (SD 4.5) Non-smokers (n=38): mean age 40.6 (SD 4.5)	Vapers (n=37): self-reported exclusive vaping product use in previous 12 months and never smoked tobacco Smokers (n=39): self-reported ≥5 cigarettes daily for previous 12 months Waterpipe users (n=40): self-reported exclusive daily use for previous 12 months Non-smokers (n=38): self-reported never use of any tobacco products People who used more than one product were excluded.	Percentage of sites with plaque: significantly higher in smokers and waterpipe users compared with vapers and non-smokers ($p<0.05$); significantly higher in vapers compared to non-smokers ($p<0.05$). PI: significantly higher in smokers and waterpipe users compared with non-smokers ($p<0.05$); no significant difference between vapers and non-smokers. BoP: significantly higher in non-smokers compared to vapers, smokers and waterpipe users ($p<0.05$) PD, CAL, MBL: significantly higher in smokers and waterpipe users compared to vapers and non-smokers ($p<0.05$); no statistically significant differences between vapers and non-smokers.	8/20

Author, year, country, study design	Participants' characteristics	Groups and definitions	Study findings	Risk of bias
Vohra, et al., 2020, Saudi Arabia, Cross-sectional (66)	n = 105, 100% male Vapers (n = 26): mean age 31.6 (SD 2.4) Smokers (n=28): mean age 33.3 (SD 2.2) JUUL users (n=25): mean age 32.1 (SD 1.7) Non-smokers (n=26): mean age 33.5 (SD 1.4)	Vapers (n = 26): self-reported use vaping product daily as sole source of nicotine intake Smokers (n=28): self-reported ≤20 cigarettes/1 pack daily JUUL users (n=25): self-reported using a least once daily as sole source of nicotine intake Non-smokers (n=26): self-reported never use of any tobacco products	PI, PD: significantly higher in smokers compared to vapers, JUUL users and non-smokers ($p<0.05$). MT, BoP, CAL, MBL: no statistically significant differences across study groups. Self-rated bad breath, gingival pain; most often reported in smokers compared to vapers and JUUL users ($p<0.001$). No difference between vapers and non-smokers. Self-rated teeth pain, gingival bleeding, bad breadth significantly higher among cigarette smokers ($p<0.001$) than non-smokers. Self-rated teeth pain, gingival bleeding, gingival pain, bad breath: no statistically significance between JUUL users compared to vapers and non-smokers.	8/20

Notes: BoP—bleeding on probing; CAL—clinical attachment loss; FU—follow-up; GI—gingival index; MBL—marginal bone loss; MT—missing teeth; OR—odds ratio; PD—probing depth; PI—plaque index; PPD—probing pocket depth; SD—standard deviation. Risk of bias assessment: ROBINS-I for longitudinal studies (low, moderate, serious, critical), BIOCROSS for cross-sectional studies (max 20 points).

Table 2. Studies in humans on ocular health outcomes and any associations with vaping

Author, year, country, study design	Participant characteristics	Groups and definitions	Study findings	Risk of bias
Kalayci et al., 2020, Turkey (17) Cross-sectional	N = 42; 100% male n=21 vaping product use, mean (SD) age=28.9 (3.4) n=21 control, mean (SD) age=28.8 (3.2)	Vapers: self-reported regular use for at least three years, liquid containing ≥ 50% propylene glycol and 3 mg/mL nicotine, ≥3 mL e-cigarette liquid a day. Control: healthy non-smokers	Foveal avascular zone significantly larger in vaping product users compared with control group (p = 0.003); total superficial and deep vascular densities significantly lower in vaping group (p = 0.012 and p = 0.041 respectively).	13/20
Makri et al., 2020, Greece (18) Acute exposure (cross-over)	N = 47; 30% (n = 14) female; mean age: 24.9 (SD=1.57, range: 23-30)	Used nicotine-containing vaping products ≥ once a week for the past 3 months and smoked cigarettes daily the last 3 months. Smoking: 1 cigarette, 10 puffs in 5 min Vaping: 18mg/mL nicotine, 10 puffs in 5 min	No statistically significant changes in subfoveal choroidal thickness, central foveal thickness and choroidal thickness between the four different conditions; choroidal vascular regulatory mechanisms remain effective in young, healthy smokers and vaping product users.	Some concerns

		Vaping: 18mg/mL nicotine, 10 puffs in 5 min followed by 25 min ad lib use Non-use: no smoking or vaping for 60 min		
Md Isa et al., 2019, Malaysia (19) Cross-sectional	N = 42; 100% male; mean age: 22.7 (SD=2)	Vapers (n=21): ≥ 1 year of vaping history who vaped at least 3 mL/d using ≥ 50% of propylene glycol in the e-liquid, had quit tobacco cigarette smoking for ≥ 6 months, or occasional tobacco cigarette smokers Control (n=21): no history of smoking or vaping	Vaping product users showed moderate-to-severe ocular surface dryness compared to healthy non-smokers; increase in vaping product voltage affects symptoms and tear instability ($p < 0.05$).	12/20
Munsamy et al., 2019, South Africa (20) Acute exposure	N = 64; 33% (n = 21) female; mean age 21	Vaped 10 puffs, 0.05 ml total, 8mg/mL nicotine	Single use vaping product exposure did not affect corneal epithelial thickness and corneal tear film ($p > 0.05$). More research is needed to determine the effect of more frequent and higher exposure.	Moderate risk

Risk of bias assessment: BIOCROSS for cross-sectional studies (max 20 points), ROBINS-I for acute exposure (low, moderate, serious, critical), RoB2 for cross-over acute exposure study (low, some concerns, high).

Table 3. Studies in humans on reproductive health outcomes and any associations with vaping

Author, year, country, study design	Participant characteristics	Groups and definitions	Study findings	Risk of bias
Harlow, et al., 2021, USA (23) Cross-sectional	N = 4,586; 100% female n=3,805 (83.0%) never users, mean age 29.8 n=609 (13.3%) former users, mean age 29.2 n= 172 (3.8%) current users, mean age 29.1	Never use: Self-reported never vaping Former use: Self-reported ever use, no current use Current use: Self-reported ever use and currently vaping >0 mL/day	Vaping product use was not statistically significantly associated with fecundability (average per-cycle probability of conception) compared to never users (ratio = 0.84, 95% CI 0.67, 1.06); the study could not account for concurrent use of vaping products and cigarettes due to inconsistent and imprecise smoking estimates.	17/20
Holmboe et al., 2020, Denmark (21) Cross-sectional	N = 2,008; median age of 19 years, 0% female n = 25 (2.1%) vaping product users n = 270 (22.0%) cigarette users n = 67 (5.5%) snuff users	Self-reported occasional or daily use	Daily vaping product or cigarette users had a significantly lower total sperm count ($p < 0.01$) compared to non-users. Testosterone level of vaping product users not statistically different from non-users, smokers had significantly higher levels than non-users No significant differences between snuff users and non-users	17/20

Risk of bias assessment: BIOCROSS for cross-sectional studies (max 20 points).

Table 4. Studies in humans on pre-diabetes or insulin resistance and any associations with vaping

Author, year, country, study design	Participant characteristics	Groups and definitions	Study findings	Risk of bias
Atuegwu et al., 2019, USA (10) Cross-sectional	N = 154,404 n = 143,952 never users, 56.9% female n = 1,339 vaping product users, 31.2% female n = 7,625 former vaping product users, 43.4% female	Never user: Self-reported never use Current user: Self-reported current daily or non-daily use Former user: Ever use but no current use	Vaping product users had higher rates of self-reported prediabetes (OR=1.97, 95% CI: 1.25-3.10) compared to never users. No statistically significant association between former vaping product users and self-reported prediabetes.	17/20
Orimoloye et al., 2019, USA (25) Cross-sectional	N = 3,415; 50.1% female, 23.1% aged 18-30, 25.8% aged 30-45, 31.8% aged 45-65 and 19.3% aged >65	Current vaping: use in previous 5 days Current smoking: Self-reported daily or non-daily smoking Dual use: current smoking and current vaping Non-use: Never smoked, no current vaping	No significant differences in insulin resistance (measured through homeostatic model assessment of insulin resistance (HOMA-IR) and glucose tolerance tests (GTT) between non-users, vaping product users, smokers and dual users. Vaping product users not significantly different from non-users.	10/20

Risk of bias assessment: BIOCROSS for cross-sectional studies (max 20 points).

Table 5. Studies in humans on allergic and asthmatic health outcomes and any associations with vaping

Author, year, country, study design	Participant characteristics	Groups and definitions	Study findings	Risk of bias
Jackson et al., 2020, USA (27) Cross-sectional	N = 99 Cohort I n = 26 non-users, 57.7% female, mean (SD) age = 33.88 (14.07) n=22 vaping product users, 54.5% female, mean (SD) age = 35.54 (12.21) Cohort II n = 25 non-users, 48.0% female, mean (SD) age = 36.2 (12.5) n = 50 cigarettes users, 50.0% female, mean (SD) age = 46.7 (10.0) n = 12 waterpipe users. 33.3% female, mean (SD) age = 33.2 (14.6) n = 10 waterpipe and cigarette users, 40.0% female, mean (SD) age = 39.5 (12.5)	Self-reported vaping, smoking, waterpipe use, dual use of tobacco cigarettes and waterpipes, or no use of any of those products	Significant increase in immune response seen by elevation of IgE levels in vaping product users compared to non-users ($p < 0.001$) but not IgG; cigarette users reported most respiratory symptoms, followed by vaping product users.	10/20
Lee et al., 2019, South Korea (26) Cross-sectional	N = 58,336; 49.2% female; mean age: 15.0 (SD=1.8) [adolescents];	Self-reported ever vaping, smoking or heated tobacco product use	Ever vaping was associated with self-reported atopic dermatitis and was not significantly associated with self-reported asthma or allergic rhinitis, unadjusted or adjusted.	17/20

Risk of bias assessment: BIOCROSS for cross-sectional studies (max 20 points).

Table 6. Studies in humans on other health outcomes and any associations with vaping

Author, year, country, study design	Participant characteristics	Groups and definitions	Study findings	Risk of bias
Boddu et al., 2019, USA (29) Cross-sectional	N = 274, 50.8% female, mean (SD) age = 30.2 (12.3) n = 126 non-users, 64.3% female, mean (SD) age = 29.4 (13.6) n = 25 cigarette users, 60.0% female, mean (SD) age = 28.5 (8.3) n = 79 vaping product users, 31.7% female, mean (SD) age = 31.8 (11.4) n = 44 dual users. 41.9% female, mean (SD) age = 30.5 (12.2)	Self-reported vaping, smoking or non-use	Sleep quality and sleep disturbances (Pittsburgh Sleep Quality Index and Leicester Cough Questionnaire) worse for female dual users compared to smokers, non-smokers, and vaping product users ($p < 0.001$).	9/20
Molino et al., 2021, USA (30) Cross-sectional	N = 441; 16.6% used vaping products in the previous year	Self-reported past-year and past 30-day vaping / smoking	Past year and past 30-day cigarette use were significantly associated with higher levels of proteinuria Occasional vaping did not represent an additional risk for kidney disease progression or risk factor for increased proteinuria or elevated blood pressure.	8/20

Kioi & Tabuchi, 2018, Japan (28) Cross-sectional	N = 4,432, aged 40 to 69, 50.8% female	Self-reported never vaper, current vaper or former vaper Self-reported never smoker, current smoker or former smoker	They found no statistically significant associations between tobacco related diseases (categorised by the authors - diabetes, asthma, cerebrovascular diseases, COPD, cancer) and either ever or current e-cigarette use, for either men or women	15/20

Risk of bias assessment: BIOCROSS for cross-sectional studies (max 20 points).

Studies in cells and animals

One in vitro study addressed the question whether tobacco or menthol flavoured vaping product affect the mouse neural stem cells, and in particular their mitochondria, by treating cells with vaping product e-liquids and corresponding aerosols of different nicotine concentration captured in cell culture media (appendices: table 7) (31). The authors concluded that vaping product exposure can produce cellular stress responses that include autophagy, but not mitophagy, dysfunction and mitochondrial hyperfusion, accompanied by oxidative stress, mtDNA damage and accumulation of calcium, with nicotine alone being able to induce these cellular responses. These observations suggest that nicotine-containing vaping product exposure may elevate the possibility of mitochondrial dysfunction and premature aging. Importantly, treatment with vaping product aerosol, a better model of true exposures from vaping product use, produced smaller responses compared to those of vaping product e-liquids.

Among the 31 in vivo studies, 11 studies (appendices: table 6) investigated the effects of vaping product aerosol exposure on the central nervous system (CNS) in rat (32) and mouse models (32-42). Vaping product exposure in rats compared with an air exposure control condition was associated with impairments in brain lipid and cholesterol homeostasis, a characteristic of many neurodegenerative diseases (32). Three studies conducted in mice revealed that chronic daily inhalation of vaping product aerosol containing nicotine compared with an air exposure control condition significantly altered homeostasis of several neurotransmitters within mesocorticolimbic brain regions, affecting dopaminergic and glutamatergic systems (37, 38, 40), which was accompanied by upregulatory effects on the nicotinic receptors α4/β2 and α7 nAChRs (38). These pathways are implicated in the rewarding and reinforcing actions of nicotine. Other research studies in mice have reported vaping product-induced neuroinflammation, disruption of blood-brain barrier integrity and impairment of memory functions (33, 36, 42) as well as decrease in brain glucose utilization (39) and other neurogenic alterations (41). Two of these studies have compared the impacts of vaping product and tobacco smoke exposures, where the vaping product group displayed similar performance to the tobacco smoke group or was uniquely affected in some contexts of memory function, such as finding the reward the next day or novel object recognition (33, 36). In addition, behavioural changes and their neurochemical correlates have been examined in withdrawal after long-intermittent exposure to vaping product and tobacco smoke, suggesting that many vaping product-induced alterations were comparable to the tobacco smoke response and in some instances the vaping product or tobacco smoke groups had significant effects by itself (34, 35). It is worth noting that the above studies used different mouse strains, device characteristics and exposure regimes that could explain some variations in the results.

Six studies (appendices: table 6) reported on outcomes relevant for digestive and reproductive systems in rats (43) and mice (44-48) following vaping product exposure

relative to air-controls. Four of them examined the effects of vaping product exposure on the mouse liver with an emphasis on DNA damage and mitochondrial dysfunction (44), oxidative stress (47), lipid metabolism and transcriptomic alterations (45). Notably, in utero exposure to vaping product without nicotine led to liver damage and altered nutrient metabolism in both pregnant mice and their offspring (46). Despite the long history of research on nicotine metabolism, understanding the role of nicotine in vaping product-induced damage is extraordinarily difficult, partially due to species-, tissue/cell type- and dose-dependent effects. Translation of findings to humans is further limited by the wide variety of commercially available nicotine products compared with the specific products used in these studies.

Additionally, harmful effects of vaping product exposure have been reported on the gut barrier in mice (48) and testis function in rats (43). While several studies demonstrated nicotine-dependent effect of vaping product exposure, others reported vaping product-induced changes with a greater magnitude in the absence of nicotine. Given that vaping product constituent variability and changes in device characteristics affect the resulting toxicant release, it is often challenging to interpret these results.

Three studies (appendices: table 6) reported outcomes on the insulin-mediated uptake of glucose with one study finding significantly decreased insulin tolerance in animals exposed to nicotine-containing vaping products (49), while 2 other studies showed no effect on insulin resistance and glucose tolerance after vaping product exposure, independent of nicotine content (25, 50). The observed differences between studies may be down to the selection of animal model and exposure methods.

Two studies (appendices: table 6) have also investigated effects of vaping products on locomotion activity, where both acute and chronic exposures to vaping product aerosol containing nicotine resulted in significantly increased locomotion in rats (51) and mice (52), respectively, with (51) also reporting decreased body temperature.

Single in vivo studies have discussed other health outcomes associated with vaping product exposure. Briefly, studies in mice reported on altered population of bone marrow hematopoietic stem and progenitor cells (53), increased urinary levels of aldehydes (54), impaired pregnancy initiation and foetal health (55), increased self-administration behaviour in response to nicotine (56), nicotine discriminative stimulus effects (57) and little or no effect on bone morphology, structure, and strength (58). While the majority of the studies conducted in mice compared the effects of vaping product exposure with air-controls, 2 studies have also investigated the effects of tobacco smoke exposure (54, 58). Furthermore, studies exposing rats to vaping product aerosol have reported on somatic withdrawal signs (59) and percentage of flap necrosis, which was increased in both vaping product and tobacco smoke groups (60). Lastly, one study using a nematode model demonstrated that vaping product aerosol did not induce cellular stress response, while tobacco smoke did (61) (appendices: table 6).

12.4 Conclusions

To address health outcomes not covered in the chapters on the main causes of smoking-related illness and death, from our systematic review we identified 15 studies in humans that looked at outcomes related to dental health. We also identified 14 studies in humans, 31 in animals and one in cells that investigated other health outcomes.

Studies in humans have assessed associations with a range of health outcomes including oral, ocular and reproductive health as well as outcomes related to allergies and pre-diabetes. The health outcomes assessed covered a limited range; all were detrimental to health and none of the included studies explored potential positive effects of nicotine or vaping. For instance, no study looked at the effects on Parkinson's disease, where some have suggested a protective effect of nicotine.

Many studies found that health outcomes for people who vaped were worse than for people who did not vape (or smoke) while others found no differences. However, while some studies included large samples, they were almost exclusively cross-sectional in design, making any causal statements impossible.

Studies used a range of different definitions of vaping and smoking. For example, findings of some studies were confounded by categorising vapers who smoke, occasional vapers or exclusive daily vapers as a uniform group or comparing occasional vapers with daily smokers. So, findings need to be cautiously interpreted. Definition of user groups, information on and comparisons with smoking were often lacking or confounded the findings. Many studies were at risk of bias and other factors (for example, genetic, lifestyle and environment) influencing health outcomes were often not considered, further limiting the validity of findings.

The evidence base on reproductive health or pregnancy outcomes remains insufficient. Previous reports found only a single study indicating that vaping in pregnancy had little or no effect on birthweight We were not able to add further evidence to these.

Oral or dental health has been researched more extensively than other health areas, however, the quality of the studies was often low. Recent reviews concluded that vaping would be detrimental to oral or dental health among people who have never vaped or smoked but would likely be beneficial for smokers switching. We found no studies that would change that conclusion.

The one cell and 31 animal studies provided insights into molecular mechanisms by which vaping products may affect the central nervous, digestive and reproductive systems as well as other target sites relative to exposure to tobacco or no exposure. However, the data are still limited and too inconsistent to evaluate the compounds of vaping product aerosol causing any alterations; variability of animal models, exposure methods and comparators added to the uncertainty.

12.5 Implications

Good quality studies in humans are needed that investigate the effects of vaping on a wider range of physical and mental health outcomes. They should also explore the progression of various health disorders in people who vape compared with people who smoke or do not vape nor smoke.

Also, although cancer, respiratory and cardiovascular diseases are the main contributors of tobacco related disease, there is a lack of research on the effects of vaping on other areas, such as renal and hepatic systems, which can be greatly affected by smoking.

Effects of vaping on foetal development and pregnancy outcomes remain in particular need of research, including the effects of switching from smoking to vaping in the perinatal phase.

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13 Poisonings, fires and explosions

13.1 Introduction

The objective of this chapter is to summarise the evidence on poisonings, fires and explosions attributed to vaping products and their component parts. We describe a summary of data from NHS Digital's Hospital Episodes Statistics about episodes of care related to tobacco or nicotine toxicity. We then present a summary of data from the UK National Poisons Information Service, American Association of Poison Control Centers' National Poison Data System and the London Fire Brigade. We also include data from a systematic review of peer reviewed literature published between 19 August 2017 and 1 July 2021.

13.2 Nicotine toxicity and poisonings

E-liquids typically consist of a solution containing propylene glycol (PG), vegetable glycerine (VG), nicotine and flavourings. This section reviews the evidence of poisoning resulting from exposure to e-liquids or components of vaping products, such as cartridges, which exceed that from routine use of vaping products. Data are presented in the following sections:

1. Data from the Hospital Episode Statistics dataset (NHS Digital) about episodes of care that were recorded as being caused by the toxic effects of tobacco or nicotine; then.

2. Data from the UK National Poisons Information Service (NPIS).

3. Data from the American Association of Poison Control Centers' National Poison Data System.

4. Findings from a systematic review of UK case reports or case series.

5. Findings from international poison treatment centres and non-UK case reports or case series.

Hospital Episode Statistics (finished consultant's episodes) in England

Table 1 includes data from the Hospital Episode Statistics (1 to 6), which contain records of all admissions, appointments and attendances for patients at NHS hospitals in England. Finished consultant episodes are the number of episodes of care for a patient under a single consultant at a single hospital (not the number of individual patients). Clinical coders

based in hospital trusts record diagnosis information using the ICD-10 classification system. From April 2015 and March 2021, there were 116,839,049 finished consultant episodes' (an average of 19,473,175 a year) which included 96,564,623 admission episodes (an average of 16,094,104 a year). Table 1 contains data about episodes of care that were coded as being caused by toxic effects of tobacco and nicotine (information specifically on vaping products is not recorded).

Of the 116,839,049 finished consultant episodes over the 6-year period, 289 episodes were recorded for toxic effects of tobacco or nicotine. The highest number of finished consultant episodes related to toxic effects of tobacco or nicotine (n=75) and admissions (n=69) were recorded in 2015-2016 and were at their lowest in 2020-2021 (n=31 and n=30 respectively). Across all years, just over half of episodes of care involved males (54.3%), patients' average ages ranged from 11 to 19 years and 48.8% of the episodes of care involved children under the age of 4 years.

Table 1. Finished consultant episodes for toxic effects of tobacco and nicotine

	2015 to 2016	2016 to 2017	2017 to 2018	2018 to 2019	2019 to 2020	2020 to 2021
FCEs for tobacco and nicotine toxicity	75	57	50	33	43	31
Admissions for tobacco and nicotine toxicity	69	52	47	32	41	30
Mean length of stay (days)	0	1	0	1	0.4	0.4
Male	43	37	22	16	20	19
Female	32	20	28	17	23	12
Mean age (years)	18	19	11	12	13	16
≤ 4 years of age n (%)	38 (50.1)	19 (33.3)	31 (62)	19 (57.6)	17 (39.6)	17 (54.8)

NPIS data, UK

NPIS is a network of dedicated poisons units commissioned by the UK Health Security Agency (formally PHE). It provides 24-hour information and advice to NHS healthcare professionals to support the management of patients with suspected poisoning across the UK (7). Information and advice are provided via 2 sources:

1. TOXBASE, an online poisons information database for which there is also an app for smartphones that works online or offline.

2. A 24-hour telephone advice service.

We previously reported telephone enquiries about suspected poisoning involving e-liquid and vaping products from 2015 to 2017 (8). In the NPIS annual report for the financial year 2018 to 2019, there were 40,466 telephone enquiries, 262 (0.6%) of which concerned vaping products. Thirty-nine percent of enquiries relating to vaping products originated from hospitals; 40% of enquiries involved children under the age of 5 years. The majority (69%) of overall exposures were accidental and 21% intentional. The remainder of enquiries (10%) were reported as adverse reactions to intended use and recreational misuse. Multiple routes of exposure occurred with ingestion being the most common (85%), and in one case e-liquid was injected. Twenty-seven exposures involved eye contact; 8 of these occurred when e-liquid was mistaken for eye drops and one was mistaken for ear drops. Of the 262 calls where the clinical features were reported at the time of the enquiry, the majority (n=177) of enquiries concerned patients who had no features of toxicity, 75 with minor toxicity, 4 enquiries concerned patients with moderate toxicity and one patient had features of severe toxicity. Features of toxicity included eye pain, oral irritation, coughing up blood, nausea, vomiting, palpitations and dizziness (9).

In 2019 to 2020, NPIS reported 38,197 telephone enquiries were received. There are no published data relating to vaping products in the 2019 to 2020 report, though there is a paragraph under ‘analysis of critical events’ stating:

> “In response to a request by the Medicines and Healthcare products Regulatory Agency, further advice on appropriate reporting of respiratory symptoms after use of e-cigarettes and other substances inhaled by vaping has been included on TOXBASE. Follow-up of these enquiries by the NPIS, where possible, has also been instituted” (10).

Colleagues at NPIS informed us that there were 182 enquiries concerning vaping products including refills, for 2019 to 2020 (stating there is the possibility that more than one enquiry might be made about the same patient).

During the 2021 calendar year, NPIS colleagues also informed us that there were 187 vaping product enquiries out of a total of 39,594 telephone enquiries made to the service, excluding mixed overdoses involving NRT. These included 108 exposures to e-liquid (including one nicotine free e-liquid), 75 reports of exposures to vaping products (including 2 nicotine free vaping products) and 4 exposures to disposable vape pens/bars. Of these, 82 involved children aged 5 years or younger. There were 8 cases recorded with moderate or severe clinical features, which involved 3 children and 5 adults. In addition, it is worth pointing out that most healthcare professional information requirements are met by TOXBASE, with the telephone service only used for a minority (for example unusual or complex cases), which therefore alone leads to substantial underestimates of the true rates of consultation. In 2021, there were 2,907 accesses to information on TOXBASE about nicotine replacement products including vaping products.

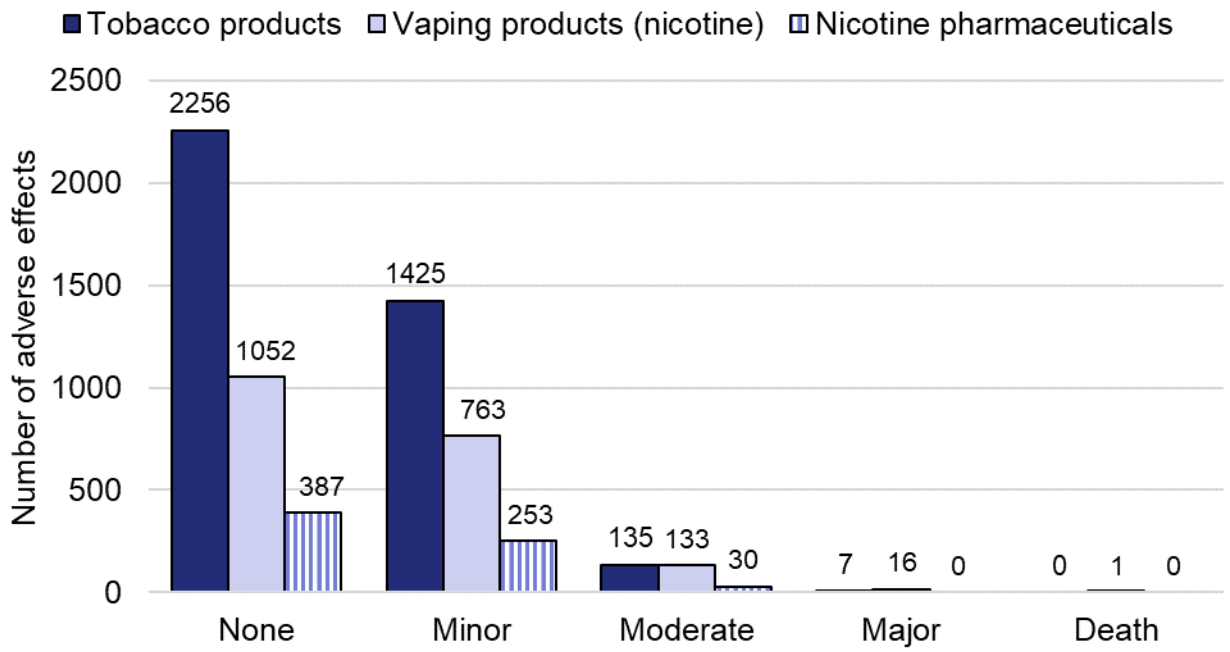
The American Association of Poison Control Centers' National Poison Data System (AAPCC-NPDS)

For comparative purposes, we also report data from the AAPCC-NPDS for the first time, which collects near real time data from all 55 national poisons centres in the US (11). Unlike NPIS, which is accessible to health professionals, the AAPCC - NPDS takes calls from public health agencies and members of the public, who can call the poison centres 24 hours a day. Poison centre staff record and upload data for every 'exposure case', rather than calls. Exposure cases are followed up and the medical outcome is also recorded. In their most recent annual report for the year 2020, the AAPCC recorded 2,128,198 exposures to pharmaceutical and non-pharmaceutical poisons. The top 3 substance classes most frequently involved in all human exposures were:

- analgesics (10.3%)
- household cleaning substances (8.37%)
- cosmetics and personal care products (6.53%)

There were 3,582 single substance exposures recorded for nicotine vaping products, 8,096 for exposure to tobacco products (for example, cigarettes, chewing tobacco, snuff) and 1,513 for nicotine pharmaceutical products. Exposures were highest for the age group 5 years and younger (n=2681 [74.8%] for vaping products, n=6844 [84.5%] for tobacco products; and n=906 [59.9%] for nicotine pharmaceutical products). The adverse effects for the 3 groups of products are displayed in Figure 1. There were more major adverse effects for vaping products (n=16) and one exposure case resulted in death (no details are given) compared with seven major adverse effects for tobacco products. Three studies included in our systematic review (tables 2 and 6) report data from NPDS up until 2019 (12 to 14).

Figure 1. Adverse effects of tobacco products, nicotine vaping products and nicotine pharmaceuticals



Source: The American Association of Poison Control Centers' National Poison Data System.

Findings from the systematic review: poisonings

When we last reviewed this topic (8), we found no peer-reviewed case reports from the UK identified by our literature search and 10 papers detailing 11 cases reported of poisonings related to e-liquids from outside the UK. Five were cases of accidental exposure, 3 involved intentional use in a suicide attempt and an additional 3 case reports, 2 of which were fatal, were unclear about the intention. A further 6 studies reported data from poison centres outside the US (8).

For this review, we searched 4 databases (CINAHL, Embase, Medline, PsycINFO) from the date we ended our search for the 2018 report (19 August 2017) to 1 July 2021 (8) (see methods, chapter 2). We included peer reviewed literature and excluded letters. We identified 22 studies related to poisonings from vaping products 2 were from the UK and 20 from outside the UK.

UK case reports

We identified 2 case reports. One described a 32 year old man who died after he was reported to have deliberatively drunk approximately 20mL from a bottle containing 72mg/mL nicotine (reported total ingestion of 1440 mg nicotine) (15). He also had signs of alcohol toxicity, though the coroner attributed the primary cause of death to nicotine

toxicity. The second case study describes a 29 year old woman who deliberately swallowed two 1.5 mL e-liquid cartridges containing 27mg of nicotine per cartridge (16). The only clinical feature was sinus tachycardia on an electrocardiogram, believed to be due to anxiety rather than leakage from the nicotine cartridge, however plasma levels of nicotine were not tested. She was observed for 72 hours, with no further ill effects and one cartridge was expelled after administering laxatives and metoclopramide.

Non-UK international poisons and surveillance centres data

We identified 12 studies that included data from poisons and surveillance centres (table 2). All studies were cross sectional or repeated cross sectional. Seven studies included children and young people ≤19 years of age (12, 17-22) and 5 included both children and adults (13, 23-26) (table 2). Seven studies were conducted in the US (12, 13, 17-19, 21, 24); 3 in Canada (20, 22, 23); one in 8 EU member states (26) and one in Czechia (25). Sample sizes ranged from 26 to 17,358, and the majority of participants were children.

Where reported, exposure was accidental in the majority of cases and the most common route was via ingestion. The most frequently reported symptoms were nausea, vomiting and dizziness. Five fatalities were reported across all 12 studies; these included 2 children (12, 19) one adult who injected e-liquid (13) and 2 where the circumstances were not reported (13, 26). Six studies reported nicotine concentrations or dose for suspected poisoning via ingestion or other routes for 0.1% to 63% of their study sample. Obertova and others. (25), was the only study to report the dose of nicotine according to body weight; among 31 cases, the median dose of ingested nicotine was 0.50mg/kg (range 0.04 to 11.25mg/kg).

Table 2. Suspected poisonings relating to vaping products (VP) reported to poison/surveillance centres: non-UK

Author, year, data source and dates	Aim of study	Sample demographics	Nicotine dose/e-liquid volume	Context of exposure	Route of exposure	Outcome
Children and young people						
Chang and others., 2019 (17) NEISS, US (2013 to 2017)	Calculate national estimates of poisoning events related to e-liquids in children under the age of 5	n=116 Age: <2, n=62; 2-4, n=54. Sex: Females n=49, males n= 67.	Reported in 46 (40%) of cases. Median nicotine dose =12mg; median volume=3.5mL	Reported for 46 cases. Mostly accidental.	Ingestion: n=111 Dermal: n=3 Other: n=1 Unknown: n=1	11 cases had info on symptoms. Vomiting, nausea, emesis n=7; crying/ eye redness n=2; cough n=1; sleepy n=1; oral cyanosis, unresponsive; hospitalised n=4
Chang and others., 2019 (18) NEISS, US (2018)	Update of above study	n=26 Age: <2, n=17; 2-4, n=9. Sex: Females n=11, males n= 15	Reported for 5 (19%) of cases: 2 cases ingested 60mL, 1 case ingested 10mL, and 2 cases ingested 0.6 mg and 3 cotton filters	21 cases reported the poisonings occurred at home.	Ingestion: n=25 Unknown: n=1	5 cases had info on symptoms. Vomiting and emesis n=5

Author, year, data source and dates	Aim of study	Sample demographics	Nicotine dose/e-liquid volume	Context of exposure	Route of exposure	Outcome
Govindarajan and others., 2018 (19) Poison Control Centres, US (January 2012 to April 2017)	Investigate exposures to VP among children <6 year and evaluate child resistant packaging legislation	n=8269 Median age: 2 years. Sex: males n=4572	Not reported	Not reported	Ingestion: n=7649 (92.5%)	Rare, severe symptoms included coma (n=4), seizure (n=4), respiratory (n=3) and cardiac arrest (n=1). 1 death (1 year old boy). Hospitalised n=115 (1.4%)
Richmond and others., 2018 (20) CPSP, Canada 12 month period, dates not reported	Explore spectrum of vaping exposure related injury among Canadian children and adolescents	n=220 (135 inhalation cases, 85 ingestion cases). Age: Inhalation cases aged 15-19. Ingestion cases: aged 1-4. Sex: 'majority males'.	Not reported	Inhalation cases: unintentional n=43; intentional n=92. Ingestion cases: unintentional n=35; intentional n=50	Inhalation n=135; Ingestion n=85	Cough, nausea, vomiting, respiratory/throat irritation, acute nicotine toxicity

Author, year, data source and dates	Aim of study	Sample demographics	Nicotine dose/e-liquid volume	Context of exposure	Route of exposure	Outcome
Rossheim and others., 2020 (21) NEISS, US (2018 to 2019) (also reported in table 6)	Estimate national rates of emergency department visits related to VP	n=45 (though reports 1555 ED visits). Median Age: 19 months. Sex: not reported	Not reported. 4 cases including marijuana or THC in the e-liquid.	Not reported	Ingestion: n=42 'e-cig in mouth': n=3	Vomiting (n=5), dizzy (n=2); choking/aspirated foreign body (n=1)
McFaull and others., 2020 (22) eCHIRPP, Canada, (April 2011 to October 2019; also reported in table 6)	Describe cases of injuries and poisonings associated VP presented to Canadian emergency departments	n=49 Age <19. Sex not reported	Not reported	Mostly accidental	Ingestion or inhalation – ns not reported	Not reported
Wang and others, 2017 (12) NPDS, US (2001-2016)	Describe trends in tobacco-related poison exposure calls involving children under 5	n=7,707 (calls involving VP) (n=83, 027 calls involving tobacco cigarettes)	Not reported	Not reported	Ingestion: n=7,108 Dermal: n=949 Inhalation: 243 Ocular: 190	Vomiting n=1218; cough or choke n=189; drowsiness n=151; eye problem n=235; oral irritation n=82; agitation n=79; other n= 85. Admitted to critical care n=42. 1 death

Author, year, data source and dates	Aim of study	Sample demographics	Nicotine dose/e-liquid volume	Context of exposure	Route of exposure	Outcome
Children and adults						
Choi and others, 2019 (23) British Columbia Drug and Poison Information Centre, Canada (2012 to 2017)	Describe epidemiological trends in VP related exposures	n=186 Median age: 3 years (range 1-75) Sex: females n=76, male n=108, unknown n=2	Reported for 97 (52.2%) of cases 0mg/mL n=4; 0.1-5mg/mL n=18; 6-17mg/mL n=15; ≥24mg/mL n=7	Accidental n=85 (45.7%).	Ingestion: n=122 Inhalation: n=28 Dermal: n=22	Self-reported symptoms: n=87 (46.8%) Asymptomatic: n=70 (37.6%) Not recorded: (n=29) 15.6%
Hughes and others, 2018 (24) Oregon Poison Center, US (July 2014 to December 2017)	Review prospective data on type of exposure, symptomatology, duration of symptoms, e-liquid concentration and flavour.	n=265 (193 children, 72 adults). Median age: 2 (range 6 months-65 years). Sex not reported	Reported for 125 (47%) of cases. Mean =14 mg/mL; median = 12 mg/mL. Range 0 mg/mL to 100 mg/mL.	Mostly accidental	Paediatrics Ingestion: n=108; Dermal: 23; Handling device 29; Inhalation: n=10; Oral mucosa: n=23. Adults Ingestion: n=23 Mucosal: 15 Ocular: n=14 Dermal: n=13 Inhalation: n=7	Paediatrics Symptomatic n=55 (28%). Most common symptoms vomiting 22/35; tachycardia 3/35. Adults Symptomatic n=55 (76%)

Author, year, data source and dates	Aim of study	Sample demographics	Nicotine dose/e-liquid volume	Context of exposure	Route of exposure	Outcome
Obertova and others, 2020 (25) Czech Toxicological Information Centre, Czechia (2012 to 2018)	Analyse the cases of acute exposure to ECs, e-liquids and heat-not-burn products reported to a national toxicology centre	n=145 (representing 0.12% of calls to national centre) Age: <18 n=92. >18 n=52, 1 unknown. Sex: females=48, males n=95, unknown n=2. 9 cases inc heated tobacco products	Reported for 91 cases (63%) Nicotine concentration: range 1 to 24 mg/mL. Volume range: 10 to 30 mL. In 31 cases, the median dose of ingested nicotine was 0.50 mg/kg (range 0.04 to 11.25 mg/kg).	Accidental: n= 110; Incorrect application: n=10; 'Abuse' n= 6; Suicide attempt= 6; Unknown: n= 16	Ingestion: n=128; Inhalation: n=9; Ocular: n=6; Intravenous: n=3	Symptomatic: n=63 (43%). Most common symptoms nausea and vomiting n=38. The dose estimation was severe/lethal in 6 (4%), toxic in 53 (36%), low-to-moderate in 35 (24%) and unknown in 54.

Author, year, data source and dates	Aim of study	Sample demographics	Nicotine dose/e-liquid volume	Context of exposure	Route of exposure	Outcome
Vardavas and others., 2021 (26) European Poison Centers from 8 EU member states (Aug 2018-Dec 2019)	Assess factors associated with EC exposures across EU member states	n=223 Age: 0-5, n=63; 6-18, n=19; ≥19: n=133 Sex: Females n=98, males n= 116	Exposure to e-liquid refills (n = 162), non-refillable EC (n=1), unknown (n=60). Nicotine concentration not reported.	All 63 incidents among children aged 0–5 years were accidental exposures to e-liquids. Abuse and misuse n=16; Suspected suicide attempt: n=8	Ingestion: n=164; Inhalation: n=34; Dermal: n=16; Ocular: n=14; Other: n=3	Symptomatic n=123 (45%). Most common nausea and vomiting n=63. Clinical outcomes, classed as moderate n=16, and major n=5, death=1

Author, year, data source and dates	Aim of study	Sample demographics	Nicotine dose/e-liquid volume	Context of exposure	Route of exposure	Outcome
Wang and others., 2020 (13) NPDS, US (2010-2018)	Describe trends and characteristics of poisoning exposure cases involving e-cigarettes and e-liquids reported to poison control centres in the US.	n=17,358 Age: ≤17, n=12,371; ≥18, n=4,110. Missing, n=877. Sex: Female, n=7648, male 9631, unknown, n=79	Nicotine concentration: Reported for 18 (0.1%) of cases, median 12mg for cases with no medical effect, 18mg for cases minor medical outcome. Volume: Reported for 64 (0.4%) cases. Median 2ml for cases with no medical effect, 3ml for minor, 30 ml for moderate medical effect.	Not reported	Ingestion: n=13,456; Dermal: n=2258; Inhalation/nasal: n=1807; Ocular: n=1232; Unknown: n=31	No effect=6068 Minor effect n=3918; moderate effect n=578; major effect n=24. Most common symptom nausea and vomiting n=3367 (19%); deaths=2 (occurred in 2012 and 2014)

Author, year, data source and dates	Aim of study	Sample demographics	Nicotine dose/e-liquid volume	Context of exposure	Route of exposure	Outcome
Children and young people						
Chang and others, 2019 (17) NEISS, US (2013 to 2017)	Calculate national estimates of poisoning events related to e-liquids in children under the age of 5	n=116 Age: <2, n=62; 2-4, n=54. Sex: Females n=49, males n= 67.	Reported in 46 (40%) of cases. Median nicotine dose =12mg; median volume=3.5mL	Reported for 46 cases. Mostly accidental.	Ingestion: n=111 Dermal: n=3 Other: n=1 Unknown: n=1	11 cases had info on symptoms. Vomiting, nausea, emesis n= 7; crying/ eye redness n=2; cough n=1; sleepy n=1; oral cyanosis, unresponsive; hospitalised n=4
Chang and others, 2019 (18) NEISS, US (2018)	Update of above study	n=26 Age: <2, n=17; 2-4, n=9. Sex: Females n=11, males n= 15	Reported for 5 (19%) of cases: 2 cases ingested 60mL, 1 case ingested 10mL, and 2 cases ingested 0.6 mg and 3 cotton filters	21 cases reported the poisonings occurred at home.	Ingestion: n=25 Unknown: n=1	5 cases had info on symptoms. Vomiting and emesis n=5

Notes: CPSP: Canadian Paediatric Surveillance Program. This conducts national surveillance on paediatric disorders or conditions, gathers information through multi-year studies and one-time surveys.

NEISS: National Electronic Injury Surveillance System. Data are collected from a probability sample of approximately 100 of the more than 5000 US hospitals with at least 5 beds and an emergency department.

NPDS: National Poison Data System. A data repository of poison exposure calls to poison control centres in the US.

eCHIRPP: Canadian Hospitals Injury Reporting and Prevention Program. An injury and poisoning surveillance system in 11 children and 8 general hospitals. Not all percentages add up to 100%. In many cases, more than one route and more than one symptom may have been reported.

Non-UK case reports

We identified a further 7 case reports including 8 people (table 3). Four reports were from Europe (27-30), 2 were from the Republic of Korea (31, 32), one from the US (33). One report was of a 6 year old who accidently swallowed e-liquid and the others were of adults aged 17 to 53, one of accidental ingestion, 4 who intentionally ingested e-liquid and one who injected it as part of a suicide attempt (table 3).

Maessen and others (34) reported a review of case reports of 31 adults from 11 countries, 23 of which were suicide attempts and 7 were accidental (one was unknown). Eleven of the cases resulted in fatalities. Mean plasma nicotine concentration was available for 5 people who died and 6 who survived; among the 6 people who survived the poisoning concentrations were 307 ± 312µg L^{-1} (median=222µg L^{-1}). Among 5 people who died, the mean plasma nicotine concentration was 3360 ± 1692µg L^{-1} (median=3000µg L^{-1}).

Summary

The systematic review identified 22 studies that reported data about poisonings related to vaping products, and 2 of these (case reports) were from the UK.

The majority of participants in the 22 papers were young children who had accidently swallowed e-liquid. Almost all children recovered (there were reports of 2 child deaths). Where exposure was intentional among adults, there were reports of 17 deaths across the 22 studies (including one in the UK). There is not much detail in these studies of the nicotine dose people were subjected to (nor any other substance in the e-liquid) or the amount of nicotine causing severe compared with mild symptoms. Maessen and others (2019) is an exception, with data on plasma concentrations resulting in severe outcomes. Where data are provided, mean (or median) concentrations are reported, which do not allow for judging the danger of intentional or unintentional ingestion of nicotine containing e-liquids. It has been estimated that the lower limit for causing fatal outcomes is 0.5g to 1g of ingested nicotine, corresponding to an oral LD50 of 6.5mg/kg to 13mg/kg (35). The only paper that included the levels of ingested nicotine according to body weight was Obertova and others. (25) who reported that in 31 cases, the median dose of ingested nicotine was 0.50mg/kg (range 0.04mg/kg to 11.25mg/kg). While it would be desirable to include information on nicotine dose ingested in fatal and non-fatal cases of nicotine poisoning, this often relies on self-report by a patient or carer and may be inaccurate or not available particularly in the case where young children are involved. Therefore, treatment should be determined by the clinical presentation of each individual case.

Most poisoning cases are preventable, underscoring the importance of regulations about child proof packaging, and including that labelling on e-liquid bottles and packaging should advise consumers to store products away from similar looking medicines such as eye drops, ear drops and children’s medicine.

Table 3. Suspected poisonings related to vaping products: Case reports – non-UK

Author, publication year, country	Cases	Nicotine dose/e-liquid volume	Context of exposure (including co-intoxication)	Route of exposure	Outcome/symptoms
Belkoniene et al., 2019 (28) Switzerland	Male, age 51	10ml of e-liquid (100mg/mL nicotine diluted in PG)	Suicide attempt	Intravenous	Patient sought treatment after 30 mins. Approximately 2 hours post-injection, became stuporous and fell into a coma. 11 hours post-injection, recovery of motor responses. Discharged after 24 hours.
Demir et al., 2018 (27) Turkey	Female, age 6	Ingested 7mL liquid containing 8.4mg (nicotine 1.2mg/mL)	Accidental; found e-liquid bottle while playing	Ingestion	Vomiting, nausea; bilateral sensorineural hearing loss that started after 24 hours and still present at 6 month follow up
Jude et al., 2021 (33) US	Male, age 21	Suspected to have drunk approx. 30m/L of e-liquid from a bottle 6mg/mL	Drank e-liquid while intoxicated	Ingestion	Severe respiratory distress and hypoxia, aspiration. Discharged within 24 hours
Maessen et al., 2019 (34) (Netherlands, literature review of case reports from 11 countries)	n=31 Females n=14, mean age 20 years; males n=17, mean age 27 years. Age range 10 months-53 years	Mean plasma nicotine concentration among the survivors (n=6) was 307 ± 312 µg L^{-1} (median: 222 µg L^{-1}). Among the 5 patients that died, the mean plasma nicotine concentration was 3360 ± 1692 µg L^{-1} (median: 3000 µg L^{-1})	Accidental (n=7), Suicide attempt (n=23), unknown (n=1)	Ingestion: n=28 Intravenous: n=2 Subcutaneous injection: n=2	Most common symptoms: tachycardia, n=18; vomiting n=13, altered mental state n=11. Deaths=11

Author, publication year, country	Cases	Nicotine dose/e-liquid volume	Context of exposure (including co-intoxication)	Route of exposure	Outcome/symptoms
McCague et al., 2018 (30) Ireland	Female, age 32	12ml of tobacco flavoured e-liquid containing 12mg of nicotine mistaken for eye lubricant	Accidental	Ocular	Corneal chemical burn with acute, pain and moderate blurry vision
Paik et al., 2018 (31) Republic of Korea	Male, age 53	The estimated amount of ingested nicotine was 450mg	Suicide attempt	Ingestion	Bradycardia, sweating, tachypnoea, and salivation. Discharged after 3 days
Park et al., 2018 (32) Republic of Korea	Male, age 27 Female, age 17	Estimated to be 23mg/kg of nicotine (male) and 30mg/kg of nicotine (female)	Both suicide attempts	Both ingested	Both patients presented seizure-like movement and cardiac arrest. They had metabolic acidosis and transient cardiomyopathy
Scarpino et al., 2020 (29) Italy	Male, age 23	2 EC refills	Suicide attempt	Ingestion	Vomiting, loss of consciousness, bradycardia, and respiratory muscle paralysis. 2 hours post ingestion nicotine plasma level was 1, 900ug/L. 4 days after ingestion severe brain oedema. 9 days after ingestion, patient died.

13.3 Fires and explosions caused by vaping products

As reported in our evidence review in 2018 (8), vaping devices and many other personal and portable electrical appliances use rechargeable lithium-ion batteries. In common with all types of batteries, lithium-ion batteries can fail. This is usually typified by a slow decline in performance to the point where the battery needs replacing. On rare occasions, a battery may fail by discharging all its stored energy at once. This can be triggered by mechanical damage, exposure to heat, water, unsafe charging, short-circuiting or by design and manufacturing faults within the battery. This type of immediate failure is known as ‘thermal runaway’ and can occur in all battery types. When thermal runaway occurs, the pressure and temperature of the battery increases and can cause the battery to vent flammable gasses at high pressure. This can cause the battery and device in which it is stored to be propelled at high velocity, resulting in a fire or ‘explosion’. This has the potential to be more extreme in lithium-ion batteries than in other types of batteries because of the large amount of energy they can store.

In our 2018 report (8), we included data about fire incidents related to cigarettes and vaping products from 41 UK Fire Services. For this report we report only on the London Fire Brigade to illustrate the number of fires caused by vaping products relative to tobacco cigarettes. The data were provided following a request under the Freedom of Information Act. Table 4 describes that between January 2017 to October 2021, there were 5,706 fires caused by cigarettes and cigarette lighters compared with 15 fires caused by vaping products. There were no fire related injuries or fatalities from vaping related fires, compared with 676 injuries and 46 fatalities from cigarette related fires. This compares with 4 fires related to vaping products in 2015 and 6 in 2016 (8).

Table 4. Fire incidents, reported by London Fire Brigade

Year	Total fires		Fire injures		Fatalities	
	Cigarettes/ cigarette lighters	Vaping products	Cigarettes/ cigarette lighters	Vaping products	Cigarettes/ cigarette lighters	Vaping products
2017	1307	4	150	0	13	0
2018	1268	2	181	0	10	0
2019	1205	2	122	0	10	0
2020	1162	3	118	0	7	0
2021*	764	4	105	0	6	0
Total	5706	15	676	0	46	0

Notes: * Data provided up to October 2021.

Findings from systematic review: injuries caused by vaping products and their batteries

When we last reviewed this topic (8) the literature search identified 25 articles, this included 3 articles describing 6 case studies of people in the UK who had sustained injuries from vaping products. In 5 cases, patients sustained burn injuries as a result of their vaping device and or battery exploding in their trouser pocket and one while charging their device. There was a further case series from a UK setting of 9 male patients who sustained superficial partial thickness and mixed depth thigh burn injuries from malfunctioning vaping device batteries. We also included 21 articles describing 43 people who had received injuries reported in non-UK case reports or case series. Most cases were patients who had sustained injuries as a result of a vaping device or separate battery exploding while being carried in a trouser pocket, in addition to other cases where the device exploded during use or while holding it. There were 3 additional articles from the international literature, one was a review of vaping related fires and explosions in the US using information reported to federal agencies, and 2 were retrospective audits of referrals to burn centres. No deaths were reported.

Our literature search for this review identified a further 25 studies that reported data about injuries related to fires and explosions from vaping products. There were 2 papers of case reports from the UK (36, 37), 7 studies that reported data from burns centres or via surveillance programmes from outside the UK (14, 21, 22, 38-41), 11 case reports from outside the UK (42-52) and 5 case series from outside the UK (53-57).

UK case reports and case series of injuries caused by vaping products and their batteries

One case report of a 19-year man (36) and a case report of 2 men and one woman, aged 16 to 29 (37) were identified from our searches. One injury occurred while using the vaping device resulting in facial, oral and upper chest injuries (36). Three injuries occurred while the vaping device was in a trouser pocket, resulting in burns to the lower extremities (37) (table 5).

Table 5. Case reports for injuries related to vaping products (VP) and their batteries: UK

Author and year	Cases	Circumstances of injury	Nature of injury	Treatment	Details of VP
La Valle et al., 2021 (36)	Male, age 19	Exploded in mouth while vaping)	Epidermal burns to face, lips and upper chest, soft tissue damage upper lip, fracture of anterior left maxilla and damage to teeth, laceration of gingivae and oral mucosa	Wound management, 4 teeth extracted. Discharged after 24 hours. No details of follow up	Photo included but no details given. Appeared to be a refillable tank.
Ho et al., 2019 (37)	Male, age 29 Male, age 26 Female, age 16	Spontaneous explosion while vaping device carried in front trouser pocket, with no other objects (males); rear trouser pocket, unsure if with other objects (female)	2-4% total body surface area (TBSA) burns to leg, buttock, hand	Wound management (bromelain based debridement). Xenograft in one case.	Reported for as re-chargeable devices with single cell, lithium-ion battery in 2 cases (males)

Data from burns and surveillance centres data of injuries caused by vaping products and their batteries: non-UK

We identified 7 studies, all from the US (table 6). Sample sizes ranged from 26 to 2035 and it is possible that some participants are included in more than one study as data sources and collection dates overlap (39, 40). All studies were cross-sectional or repeated cross-sectional. Ages ranged from under 5 years to over 60 years. The majority were male in 4 studies (22, 38, 40, 41), evenly balanced in one (14) and not reported in 2 studies (21, 39). The circumstances of the cause of the injury were missing in most studies and, where reported (21, 39, 41) people had been carrying their vaping device or battery in their trouser pocket, either on its own or with other metal objects. The most common injury was thermal or chemical burns to a thigh, but also to other parts of the body such as hands, while trying to extinguish the fire, abdomen and face. Information about treatment outcome was missing from most

studies. Where reported, the minority of cases were hospitalised and no deaths were reported.

Case reports of injuries caused by vaping products and their batteries: non-UK

Eleven case reports were identified, 10 were from the US (42 to 49, 51, 52) and one from Ireland (50) (table 7). Cases described 10 males aged between 17 and 53 years of age and one female aged 30. The vaping device had exploded and caught fire either while holding it (n=5), while in the mouth (n=3), or in the person's trouser (n=1). Burn and/or bone injuries were sustained to the hand (n=4), face, inside and outside the mouth (n=5), thigh and calf (n=1) and breast (n=1). In addition to the management of wounds, treatment involved grafts in 6 cases and oral surgery in 5 cases. One of the 11 cases was a 38 year old man who was found dead at home following a fire. He reportedly died when his vaping device had exploded and propelled itself through his upper lip and the whole device lodged itself in his cranium. Eighty per cent of his body was also covered in burns. Details were provided about the type of vaping device in this case and another case (44, 51) - both were mechanical mod devices.

Case series injuries caused by vaping products and their batteries: non-UK

Five case series including 54 people were identified from burns centres of surveillance centres in the US (n=2, (54-56), Canada (n=1), France (n=1), (53) and Germany (n=1), (57) (table 7). Ages ranged from under 4 to 50 years, and only 2 females were included. In most cases the vaping device or separate battery exploded and/or caught fire in a trouser pocket (n=51, 88%), in the hand in 5 cases, in a purse in one case and there was a case of a child swallowing part of the device. The total body surface area burned ranged from 0.5% to 10%. Most injuries were to a thigh, and also included burns to the hands, abdomen, genitals, buttocks and lower leg. Twelve patients required skin grafts. There were no reports of deaths.

Summary

Of the 25 studies that reported data about injuries related to fires and explosions from vaping products, 2 case reports were from the UK. Most studies involved injuries to men, and only 2 women were included. Where reported, the majority of explosions happened while people carried their device or separate battery in their trouser pocket, and it followed that the majority of the injuries were to the thighs. A minority of reports suggested the vaping device/battery was in a trouser pocket along with other metal objects such as keys and coins. For the incidents we included in this chapter, possible explanations have been offered by some authors as to why this issue may largely affect men. There are 2 separate triggers hypothesised for

‘thermal runaway’ reactions. The first is an exothermic reaction between the lithium and moisture (such as the moist environment created by perspiration in a trouser pocket), resulting in the formation of lithium hydroxide and hydrogen (37, 58). The second trigger may be due to the production of a short-circuit by metallic objects commonly found in pockets, such as keys, causing the battery to overheat (37). Men may be more likely to carry their device in their trouser pocket, whereas women may be more likely to carry their device in a bag.

Very little detail was provided about the type of device involved in the incidents. Satteson and others. (51) and Beining and others. (44) indicated mechanical mods were the cause of 2 incidents, one of which was fatal. These types of devices are built by the user and do not have any inbuilt safety features, unlike other types of vaping devices. In recent years, vaping websites, including some online sellers, have marketed these devices at very experienced vapers and often (but not always) advise that potential users should have knowledge of vaping hardware and novice vapers should be discouraged from using them.

The Chartered Trading Standards Institute and Office for Product Safety and Standards in the UK (59) have recently run a campaign to highlight the risks of incorrect use of batteries and chargers used for vaping devices. Future campaigns could include general advice about carrying devices in pockets, not just a warning about carrying them with metal objects.

Table 6. Data on injuries related to vaping products (VP) and their batteries from burns and surveillance centres: non-UK

Author, year of publication, dates of data collection	Sample demographics	Circumstances of injury	Details of injury	Treatment/outcome
Dohnalek et al., 2019 (38) NEISS, US (2008 to 2017)	n=49 Age: <18: n=3, 18 to 29: n=26, 30 to 44: n= 14, 45 to 60: n=5, >60. Sex: Females n=2, males, n=47	Not reported	Injury to upper leg n=29, (59%), hand n=8, lower abdomen n=4, lower arm n=3, head n=2, lower leg n=2, shoulder n=1	Hospitalised n=13
Corey et al., 2018 (39) NEISS, US (2016)	n=26 Age: <18: n=3, 18 to 24: n=4, 25-55: n=19. Sex: not reported	20 injuries occurred while the battery from the VP was in the user's pocket	The most common burn type was thermal burns (80.4%). Injuries to the upper leg/lower trunk (77.3%), hand or lower arm (19.7%), other body parts (3.1%)	Hospitalised =26%
Flores et al., 2021 (41) American Burn Association registry	n= 27 Mean age: 34 (range 1-75). Sex: Female (n=14, male n=113	While using the device 78% (n=100); spontaneous combustion 18%; while changing the battery 1.5%, while modifying the device 1.5% 9 (does not report ns for holding the device or had it in their mouth, though reports most had it in their pocket)	<10% TBSA (mean 3.8%, range 0.1 to 16.5%). Thermal burns n=45; thermal and chemical =37. Injury site: Head and neck 15%; torso 9.5%; right hand and arm 30.7%; left hand and harm 27.6%; right thigh, calf and foot 40.9%; left thigh, calf, foot 40.2%; genitals 11%. No deaths	Hospitalised n=92 (72%); mean length of hospital stay 6.7 days. Surgery n=46; topical antimicrobials n=118; debridement n=20

Author, year of publication, dates of data collection	Sample demographics	Circumstances of injury	Details of injury	Treatment/outcome
McFaull et al., 2020 (22) Hospitals Injury Reporting and Prevention Program, Canada (Apr 2011 to Oct 2019)	n=4 Age range: <4 years -49 years. Sex: All male	While disassembling device (n=1); swallowed piece of device (n=1); battery exploded while in trouser pocket (n=2)	Crushing injury to finger (n=1); foreign body in alimentary tract (n=1); thigh burn (n=2)	Not reported
Rossheim et al., 2019 (40) NEISS, US (2015 to 2017)	n=2035 Median age: 26 years. Sex: Females 6%, males 94%	Not reported	Majority were burns (97%), to upper leg (61%) or hand/fingers (25%)	Treated and discharged within same appointment =69%; admitted hospital = 26%; left without being= seen 5%
Rossheim et al., 2020 (21) NEISS, US (2018 to 2019) [also reported in table 1]	n = 676 Mean age 37.1 range 13-66) (for 2019). Sex: not reported	Reported for 20 cases. While carrying in pocket: n=19 (including 1 with coins in pocket and 1 when fell on it) 1 while holding VP	Not reported	Not reported

Author, year of publication, dates of data collection	Sample demographics	Circumstances of injury	Details of injury	Treatment/outcome
Wang et al., 2020 (14) NPDS, US (2010 to 2019)	n=69 Age: ≤5 years 2%, 12-17 year 11.5%, 18-25 years 29%, ≥25 years 43.5%, unknown 13%. Sex: Females 53.5%	'Involved explosion' n=45, circumstances not reported	Where reported, type of burn: thermal n=43, chemical n=21, both n=5. Site of injuries: face n=23, leg/thigh n=13, hands n=10, chest n= 1, genitals n=1, more than one body part n=18. 2 cases had life-threatening injuries	Hospitalised n=4, treated, and released n=45

Notes: NEISS: National Electronic Injury Surveillance System. Data are collected from a probability sample of approximately 100 of the more than 5000 U.S. hospitals with at least 5 beds and an emergency dept.

NPDS: National Poison Data System. A data repository of poison exposure calls to poison control centres in the US. Not all percentages add up to 100%. In many cases more than one injury or symptom may have been reported.

Table 7. Case reports for injuries related to vaping products (VP) and their batteries: non-UK

Author and year	Sample demographics	Circumstances of injury	Nature of injury	Treatment
Case reports				
Ackley et al., 2018 (42) US	Male, age 17	VP exploded in hand	Soft tissue damage to thumb and joint.	Wound debridement and management. Lateral resection of thumb
Ban et al., 2017 (43) US	Male, age 53	VP exploded after changing battery, in hand	Fracture to thumb, part of device embedded in maxilla First degree burns to his upper lip, soft palate	Wound debridement and management, maxillary graft 8 months post-injury
Beining et al., 2020 (44) US	Male, age 38	Found dead at home with flames partially covering the room. Mechanical mod	Vaping device (whole) in cranium, entered via philtrum region of upper lip	80% burns to body. Died from traumatic brain injury
Chi et al., 2018 (45) US	Male, age 20	VP exploded in mouth shortly after charging battery	Orofacial trauma inc tooth fracture, burns and lacerations; granulated wounds of labial mucosae	Tooth extraction, wound management, lost to follow up.
Foran et al., 2019 (46) US	Male, age 30	VP exploded in hand	Trauma to hand; first and second-degree burns to hand, with deposition of subcutaneous radiopaque debris	Wound management, granuloma/cyst excision, skin graft after 5 months
Hagarty et al., 2020 (47) US	Female, age 30	VP exploded in mouth shortly after replacing battery. Modified vaping product	Mixed partial and full thickness burn with laceration on lower inner lip; tongue laceration; soft tissue and dental injury, C1 fracture and left vertebral artery dissection	Surgery for C1 fracture treated with C-collar; wound management, tongue and oral mucosa reconstruction

Author and year	Sample demographics	Circumstances of injury	Nature of injury	Treatment
Katz et al., 2019 (48) US	Male, age 17	VP exploded in mouth while vaping	Facial trauma including circular puncture to chin, mouth lacerations, teeth loss and bone damage, mandibular fracture	Open reduction and internal fixation of fracture, dental extraction, tissue debridement
Michael et al., 2019 (49) US	Male, age 40	Exploded while in trouser pocket. Photo of burned tank style device included	Severe burns to thigh and calf. Antalgic gait with external rotation of the lower leg and foot (limp) after 1 month	Wound management and skin graft.
Quinlan et al., 2020 (50) Ireland	Male, age 60	E-liquid had leaked from the vaping device while carrying it in breast pocket	0.3% Total Body Surface Area (TBSA); full thickness chemical burn to left breast	Wound management and skin graft
Satteson et al., 2018 (51) US	Male, age 35	Exploded in hand shortly after changing battery. Dark Horse atomizer with SMPL Mec Mod battery	Blast injury with mixed partial and full thickness burns to thumb and palm of hand	Wound management, carpal tunnel release, removal of radiopaque material, reconstruction of thumb radial digital artery, nerve grafting, required 10 surgeries and treatment for up to 15 months
Vaught et al., 2017 (52) US	Male, age 20	While holding and switching the device on, exploded and propelled the mouthpiece into his face. Battery started a fire several feet away	Soft tissue injury over right nasal bone, fractures (naso-orbital-ethmoid, frontal sinus, maxilla), obstruction of sinus outflow and pneumocephalus	Surgical repair (antrostomy, ethmoidectomy, reconstitution of sinus) with placement of steroid stent.

Author and year	Sample demographics	Circumstances of injury	Nature of injury	Treatment
Case series				
Boissiere et al., 2020 (53) Montpellier University Hospital Burn center, France (April 2014 to May 2019)	n=16	All the patients were carrying their e-cigarette or battery in their pants pocket and one patient in the jacket pocket. All patients described the presence of flame, with overheating before the fire in half. of the cases. 9 patients reported battery was in contact with other objects in their pocket (keys or coins)	All cases included thermal burns and 75% chemical burns to either thigh, buttocks, genitals, trunk. Average TBSA was 5%.	Surgical management with excision and split-thickness skin graft n=6, others healed spontaneously in several weeks following wound management.
Gibson et al., 2019 (54) Electronic Medical Records of 1 hospital, Oregon US (2012 to 2016)	Mean age: 41 years	Exploded in pocket n=12, in hand n=2	<1% to 6% TBSA mixed partial and full thickness burns to thigh n=6, thigh and hand n=6, hand and lip n=1, hand n=1	Skin graft n=3. Wound management. Average time to recovery 24.5 days

Author and year	Sample demographics	Circumstances of injury	Nature of injury	Treatment
Hickey et al., 2018 (55) General Hospital Burn Center, Massachusetts, US (January 2015 to April 2017)	Sex: all Male	While in trouser pocket n=12, hand n=1, purse n=1. Mean length of time patient started using ECs prior to burn (years) (n=7) 1.91.2, (0.17 to 4)	4.7 ± 2.4% (range 1-10%) TBSA second- and third-degree burns to thigh and/or leg n=9, thigh and buttock n=2, thigh and genitals n=2, hand n=1	Skin graft n=8. Wound management. Average length of stay 6.6 days, (0 to 15)
Quiroga et al., 2019 (56) Johns Hopkins Burn Centre, US	n=14	Vaping device or battery on its own exploded in trouser pocket in all cases	2% to 6% TBSA to thigh and hand	Wound management. 1 patient required excision and graft
Welter et al., 2020 (57) Germany	Age range: 16-49 years. Sex: Female n=1, males n=13	3 cases involved explosion of VP in trouser pocket, 1 case exploded in hand	0.5% to 4.5% TBSA, injuries to thigh, hand, abdomen and genitals.	Wound management and surgery

Quality of studies

Case reports, case series and cross-sectional studies were assessed using the Joanna Briggs checklists and details can be found in the appendices. Overall, the quality of the case reports was good, and that of the case series and cross-sectional studies variable.

As discussed in our 2018 report (8), case reports and case series have long been accepted as a way to present unusual, uncontrolled observations regarding symptoms, clinical findings and novel treatments and are often written to educate other clinicians. They can alert us to precautions that can be taken to minimise further events and guide clinical treatment decisions. However, as a methodology, they are limited; they are not chosen from representative population samples and cannot be generalised. They rely on the patients' recall of events and the observer's subjectivity. There is a bias towards over-representation of severe cases, and they often report rare and atypical events and can be easily over-interpreted or misinterpreted, as they often have an emotional appeal on readers, particularly when accompanied by graphic images of injuries. The studies included in this chapter cannot provide information on incidence or prevalence of poisoning, fires or explosions and cannot be generalised to the current approximately 3 million vapers in England.

13.4 Conclusions

Poisonings

In 2021, the National Poisons Information Service reported the service received 187 vaping product enquiries out of a total of 39,594 telephone enquiries. Of these, 82 involved children aged 5 years or younger. This equates to at least every other day NPIS having a telephone enquiry involving a healthcare professional managing an individual who has apparently been exposed to vaping products.

Two case reports of poisoning from vaping products in the UK were identified, both intentional. In one of the cases, the person died.

In non-UK poisonings, according to data from a 2020 annual report by the American Association of Poison Control Centers' National Poison Data System, one person died from the use of a vaping product (no details were given of the circumstances). In 20 studies from international poisons/surveillance centres and case reports/studies identified in a systematic review, the majority of participants were young children who accidentally swallowed e-liquids. Almost all children recovered, although there were 2 fatalities among the children who were accidentally exposed to e-liquid. Where exposure was intentional or unknown, there were reports of 16 deaths (outside the UK).

Accidental ingestion is the most common cause of poisonings, with fewer incidences of other routes such as ocular exposure.

Incidents of poisoning in children are often preventable.

Fires

Between January 2017 and October 2021, there were 5706 fires caused by cigarettes and cigarette lighters compared with 15 fires caused by vaping products, reported by the London Fire Brigade. No fire related injuries or fatalities were reported from vaping related fires, compared with 676 injuries and 46 fatalities from cigarette related fires. These findings are similar to those we discussed in our 2018 report.

Explosions

Exploding vaping products can cause severe burns and injuries that require intensive and prolonged medical treatment especially when they explode in users' hands, pockets or mouths.

Incidents appear to be serious but very rare.

Two case reports involving 4 people in the UK were identified. One involved an explosion in the mouth while vaping, the other 3 involved explosions when the vaping product was being carried in trouser pockets. No fatalities were reported.

There were 23 reports identified outside the UK, from case reports/series or data from burn/surveillance of injury centres. Carrying the vaping product in a trouser product was again the most common cause of explosions. One fatality was reported.

13.5 Implications

There is a dearth of UK research or published case reports. The findings reported here are largely from the US and cannot be assumed to be generalisable to the UK given the different regulatory frameworks for vaping products.

Information on poisonings, fires, and explosions should be monitored and reported routinely in publicly available reports by relevant authoritative bodies.

More research is required on the type of vaping product resulting in poisoning, fires and explosions, which would then inform future regulations.

Two explosions were identified as caused by mechanical modifiable tank devices which do not have inbuilt safety features, so warnings could be highlighted for users of these products by relevant authoritative bodies.

In addition to childproof packaging, regulations should require labelling to reinforce safe storage, away from similar looking medicines such as eye or ear drops and children's medicine.

Additional advice by relevant authoritative bodies could be given on transportation of vaping products and batteries, to avoid thermal runaway incidents (where a battery discharges all its stored energy at once), for example in specialised containers.

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14 Heated tobacco products

14.1 Introduction

The objective of this chapter is to summarise available data on the use of heated tobacco products (HTP) in youth and adults in England, and a recent Cochrane review (1) evaluating the effectiveness and safety of HTP for smoking cessation and the impact of HTP on smoking prevalence.

The most widely used HTP are composed of a device that contains an external energy source and an insert containing processed tobacco. The device then heats the tobacco to temperatures typically less than 350ºC to release an aerosol (2). They differ from e-cigarettes because they heat tobacco leaf or sheet rather than a liquid.

In England, HTP are regulated under the Tobacco and Related Products Regulations 2016 as ‘novel tobacco products’ (3). As such, HTP do not fall under regulations requiring standardised packaging or graphic health warnings. Age of sale and advertising regulations apply and there is a requirement to notify the competent authority (the tobacco team in the Office for Health Improvement and Disparities) at least 6 months before a product is supplied, providing information such as a description of the product, mechanisms, ingredients, emissions, nicotine absorption and available studies on toxicity, addictiveness and attractiveness.

For duty rate (tax) purposes, following a consultation, England decided to treat tobacco for heating separately from cigarettes, cigars, hand-rolling tobacco and other tobacco products. It was initially (July 2019) taxed at the same rate as hand-rolling tobacco, but with a differential built in which means that HTP tax will increase more slowly than tax for hand-rolling tobacco. As of 27 October 2021, tobacco for heating has a duty rate of £270.22 per kg. For comparison, the rate for hand-rolling tobacco is £302.34 per kg and for cigarettes it is £262.90 per 1,000 cigarettes plus 16.5% of the retail price (4).

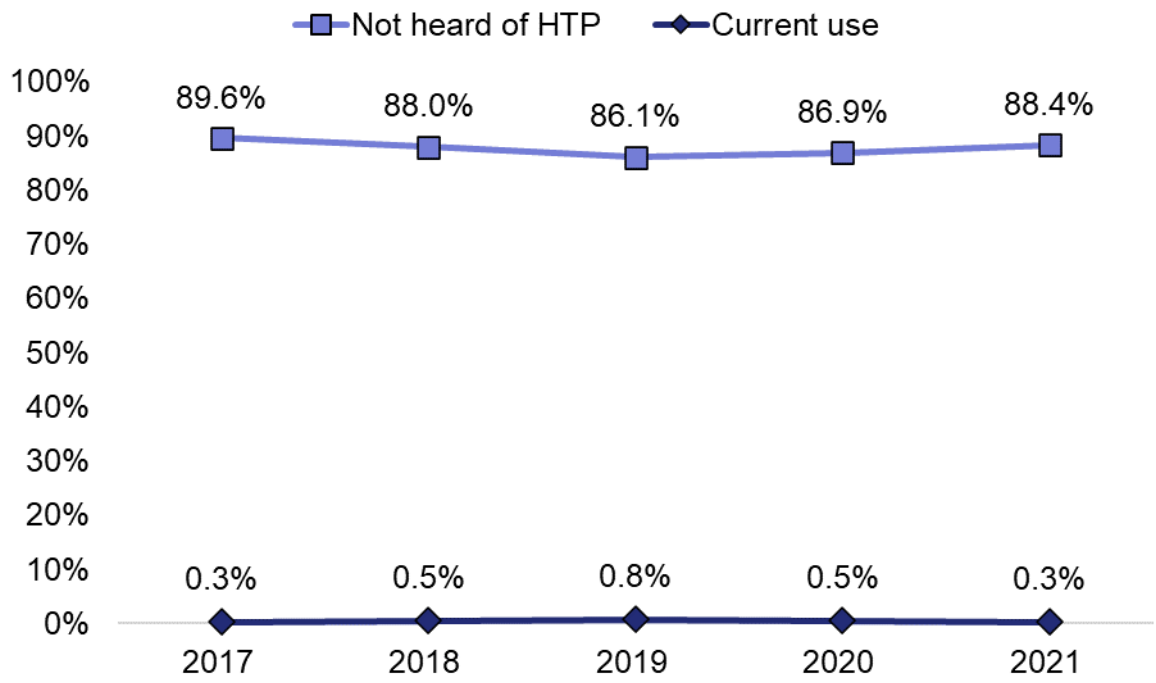
The World Health Organization recommends that heated tobacco products, including the devices, are regulated and taxed in the same way as tobacco for smoking (2).

In our 2018 report we gave an overview of the HTP market in England and systematically reviewed the literature on HTP emissions and use (5, 6). Since then, our annual reports in 2019, 2020 and 2021 (7-9) have indicated low use of HTP in England, also reported in the peer reviewed literature (for example, (10-12)). In this report, we report recent data on HTP use from the surveys used in chapter 3 (vaping among young people) and chapter 4 (vaping among adults), which were ASH-Y and ITC Youth, and the STS and ASH-A surveys. This chapter also summarises the recent Cochrane review on HTP (1).

14.2 HTP use among young people in England

Most participants in the ASH-Y survey in 2021 (88.4%) had not heard of HTP (Figure 1). Awareness was lower among younger respondents and higher among those who were currently smoking or vaping or who were former users or triers (table 1). Ever use of HTP was rare among 11 to 18 year olds with 0.9% in the ASH-Y reporting they had tried them but no longer used them and 0.3% reporting current use (table 1). The number who had ever used them was too small to allow breakdowns by socio-demographics, smoking or vaping status (both groups combined had unweighted count of 26 participants). Awareness and current use have varied little since 2017 when this question was first asked (Figure 1).

Figure 1. Percentage of young people in England who have not heard of HTP and who report current use of HTP, England 2017 to 2021 (ASH-Y, weighted data)



Notes: The survey used the term 'heat-not-burn'. Unweighted bases: 2017 = 2,260; 2018 = 2,011; 2019 = 2,173; 2020 = 2,168; 2021 = 2,151

Among 16 to 19 year olds in the ITC survey, 13.5% had heard of HTP before the survey and 2.2% had ever used HTP, including 0.7% who had used HTP in the past week. Of those who had ever used HTP, 27.5% reported trying HTP once, 38.2% trying HTP 2 to 10 times, 27.0% reported use between 11 and 99 times, 2.5% reported use at 100 or more times (unweighted n=4 out of 4298) and 4.8% did not know or refused to answer this question.

Table 1. Awareness and use of heated tobacco products among young people aged 11 to 18 by age, gender, region, social grade, smoking and vaping status, England 2021 (ASH-Y, weighted data)

	Never heard of %	Heard of but not tried %	Tried but do not use anymore* %	Tried and still use them* %	Don't know %
Total	88.4	5.4	0.9	0.3	5.0
Age					
11 to 15	90.1	4.0	-	-	5.0
16 to 17	87.0	7.1	-	-	4.6
18	83.0	9.0	-	-	5.5
Gender					
Female	87.7	5.6	-	-	5.5
Male	89.1	5.2	-	-	4.6
Region					
North	89.9	4.3	-	-	5.0
Midlands	91.1	3.3	-	-	4.7
South	86.6	6.9	-	-	5.2
Social grade					
ABC1	88.7	5.8	-	-	4.1
C2DE	87.6	4.4	-	-	7.2
Smoking status					
Never	92	3.4	-	-	4.1
Tried only	78.3	14.8	-	-	5.3
Former	72.7	9.1	-	-	10.6
Current	58.4	22.5	-	-	7.9
Vaping status					
Never	93.5	3.5	-	-	2.9
Tried only	82.6	11.1	-	-	5.3
Former	73.1	11.5	-	-	7.7
Current	48.3	26.4	-	-	13.8

Notes: The survey used the term 'heat-not-burn', Unweighted base = 2,151. Never smokers were people who had never tried cigarettes. Tried only smokers were people who had only ever tried smoking cigarettes once. Former smokers were people who used to smoke sometimes but who never smoked now. Current smokers were people who smoked sometimes but less than weekly, as well as those who smoked more than once a week. Never vapers were people who had never tried vaping. Tried only vapers were people who had only tried vaping once or twice. Former vapers were people who used vaping products in the past but who no longer do. Current vapers were people who vaped at least monthly.

*Columns with fewer than 50 participants have not been broken down as they do not represent a wide enough cross-section of the target population to be considered statistically reliable.

14.3 HTP use among adults in England

The STS survey asks about current use of HTP for any reason and ASH-A asks about trying HTP and whether people still use them. Prevalence of HTP use among adults has remained low at 0.3% in the STS and 0.5% in the ASH-A survey (table 2).

Table 2. Use of heated tobacco products among adults (18+) by year, England 2017 to 2021 (STS and ASH-A, weighted data)

Year	STS %	ASH-A %
2017	0.1	0.8
2018	0.1	0.4
2019	0.1	0.6
2020	0.2	0.3
2021	0.3	0.5

Notes: STS: unweighted bases 2017 = 20,394; 2018 = 20,702; 2019 = 20,641; 2020 = 18,513; 2021 (January to September) = 14,882. Included current use of heated tobacco products for any reason. 2021 data available from January to September.

ASH-A: unweighted bases 2017 = 10,487; 2018 = 10,578; 2019 = 10,208; 2020 = 9,329; 2021 = 10,211. Included people who have tried heated tobacco products and still use them.

In the STS, ever or past use of HTPs is not reported, and no further breakdown or analysis was undertaken due to only 39 adults reporting current use in 2021.

Breaking down 2021 ASH-A estimates of awareness, trial and use by socio-demographics, smoking and vaping status suggests some variations (table 3). Awareness and trial appeared higher among women and younger adults and there were higher rates of awareness, trial and use among former and current smokers and former and current vapers. With prevalence overall very low, subgroups of trial and use are based on small numbers, so caution is needed when interpreting these figures.

Statistical testing using ASH-A data compared rates of ever use of HTP (any trial or use, overall 1.8%) by demographics and smoking or vaping status. This indicated differences in ever use by age, with 25 to 34 year olds most (3.9%) and those aged 55+ least (0.4%) likely to have ever used HTP (χ^2=114.2, p<0.001). Women (2.4%) were more likely to have ever used than men (1.2%, χ^2=21.8, p<0.001), adults who currently smoked were most (6.6%) and never smokers least (0.6%, χ^2=238.7, p<0.001) likely to have ever used HTP. Similarly, those who currently vaped were most (7.1%) and those who had never vaped least (0.6%, χ^2=321.4, p<0.001) likely to have ever used HTP.

The STS also reports use of HTP in the most recent attempt to stop smoking. In 2021, HTP were used in 1.6% of smoking cessation attempts (unweighted n=15) among past year smokers who had attempted to stop smoking.

Table 3. Awareness and use of HTP among adults by age, gender, region, social grade, smoking and vaping status, England 2021 (ASH-A, weighted data)

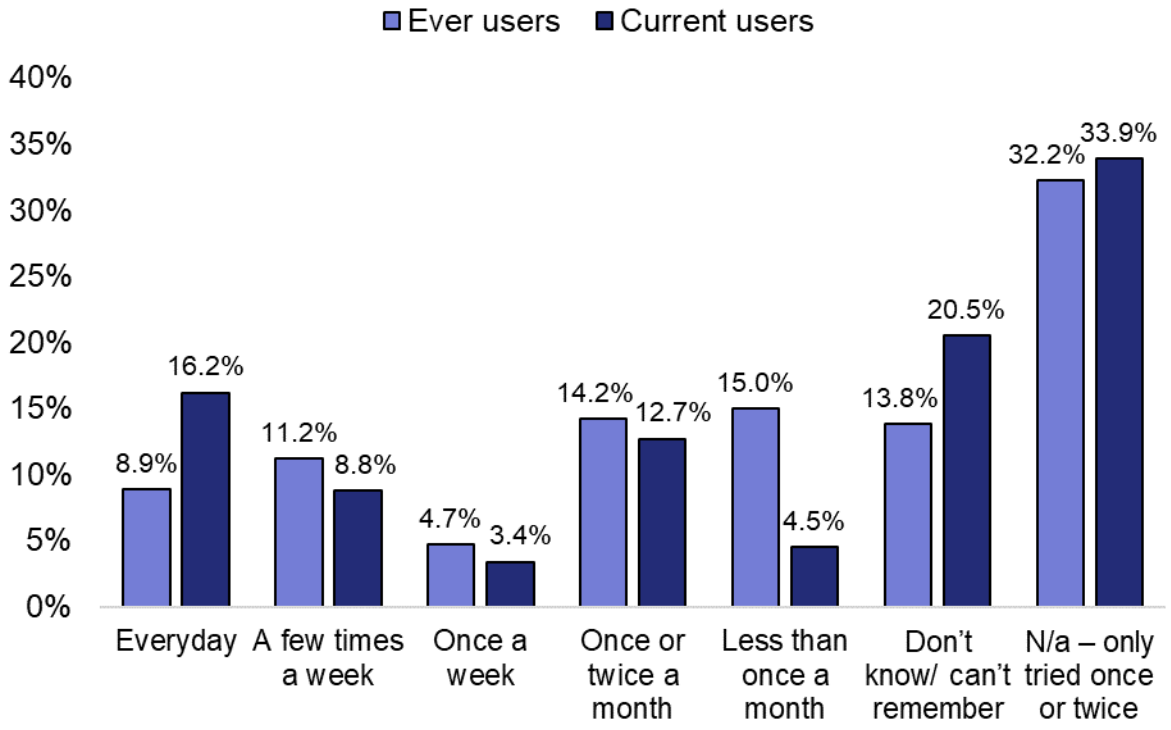
	Never heard of %	Heard of but not tried %	Tried but don't use anymore %	Tried and still use them %	Don't know %
Total	81.4	12.2	1.2	0.5	4.6
Age					
18 to 24	75.9	14.2	2.1	1.1	6.8
25 to 34	74.9	13.9	3.3	0.6	7.2
35 to 44	77.3	15.2	1.4	1.1	5.0
45 to 54	79.5	14.8	0.7	0.6	4.4
55+	88.0	8.6	0.3	0.1	3.0
Gender					
Female	75.8	16.4	1.7	0.7	5.4
Male	86.7	8.2	0.8	0.4	3.9
Region					
North	83.5	10.5	1.1	0.5	4.4
Midlands	83.4	10.3	1.0	0.6	4.7

	Never heard of %	Heard of but not tried %	Tried but don't use anymore %	Tried and still use them %	Don't know %
South	78.7	14.6	1.5	0.5	4.7
Social grade					
ABC1	82.2	12.3	1.3	0.5	3.6
C2DE	80.5	12.0	1.1	0.6	5.8
Smoking status					
Never	86.3	9.0	0.4	0.2	4.1
Former	82.1	12.4	1.2	0.6	3.6
Current	59.3	24.7	4.9	1.7	9.3
Vaping status					
Never	86.5	9.9	0.4	0.2	3.1
Former	67.5	20.1	4.3	1.2	6.9
Current	63.2	22.1	4.2	2.9	7.6

Notes: unweighted n=10,211. Vaping status n=10,101 (n=110 who did not know their vaping status excluded).

When asked about frequency of use, the modal response both for ever users and the subgroup of current users was that they had only tried HTP once or twice (Figure 2, ASH-A). Among current users, 21% were unsure about their frequency of use and 16% used HTP daily, which means that less than 0.1% of adults in England reported daily HTP use in 2021.

Figure 2. Frequency of HTP use among ever users and the subgroup of current users, England 2021 (ASH-A, weighted data)



Notes: ever use unweighted n=152, current use unweighted n=48.

14.4 Cochrane review: heated tobacco products for smoking cessation and reducing smoking prevalence

As reported in the previous section, a small minority (1.6%) of adults attempting to quit smoking use HTP as support in their most recent quit attempt.

A recent Cochrane review (1) aimed to evaluate the effectiveness and safety of HTP for smoking cessation and the impact of HTP on smoking prevalence. It included randomised controlled trials (RCTs) in which people who smoked cigarettes were randomised to switch to exclusive HTP use or to a control condition, and time-series studies that examined the population-level impact of HTP on smoking prevalence or cigarette sales.

Primary outcomes were a) tobacco smoking cessation at the longest follow-up point available, using intention-to-treat and biochemically verified abstinence where possible, b) safety reported as number of people reporting adverse events and serious adverse events and c) smoking prevalence. Secondary outcomes assessed safety using biomarkers of toxicant and carcinogen exposure such as tobacco-specific N-nitrosamines (NNAL), polycyclic aromatic hydrocarbons (1-OHP, 1-Naphthol, 2-Naphthol), volatile organic compounds (3-HPMA, MHBMA), and carbon monoxide (COHb and exhaled CO).

Secondary outcomes further included biomarkers of harm to lung and cardiovascular function (also known as surrogate endpoints) such as FEV1, FVC, FEV1/FVC, blood oxygen saturation, systolic and diastolic blood pressure and heart rate.

The literature search was conducted in January 2021 and methods followed Cochrane guidance (13). Sensitivity analyses removed studies judged at high risk of bias for at least one domain, studies with a minimum follow-up length of less than 4 weeks and studies that used carbon rather than electronically heated HTP. For biomarkers, subgroup analyses investigated differences for individual study analyses using intention-to-treat versus per-protocol analyses.

Thirteen studies were included in the review; 2 were interrupted time-series studies using sales data from Japan and 11 were RCTs assessing safety with a total of 2,666 participants. All 11 RCTs were funded by tobacco companies, 8 were judged to be at unclear risk of bias and 3 at high risk. All RCTs compared participants randomised to use a HTP or to continue smoking cigarettes. Five RCTs had a tobacco abstinence group as an additional comparator and one trial had an additional comparator group of snus use (1), which will be omitted for this summary.

Effectiveness for smoking cessation

No studies reported on HTP use for cigarette smoking cessation, so the effectiveness of HTP for stopping smoking remains uncertain (1).

Toxicant and carcinogen exposure

Pooled data from RCTs indicated lower exposure to NNAL, COHb, 1-OHP, 3-HPMA, MHBMA and exhaled CO in groups using HTP compared with cigarette smoking (table 4). Compared with abstinence, NNAL, 1-OHP, 3-HPMA, MHBMA were higher in groups using HTP. For COHb, results were inconsistent, showing higher COHb in HTP for intention-to-treat analyses and lower COHb for per-protocol analysis (defined as only including participants who exclusively, or almost exclusively, used the assigned product; table 4). Heterogeneity was high for all outcomes with the exception of NNAL (low heterogeneity) and 1-OHP (moderate) in the HTP versus abstinence comparison. However, the direction of difference was generally consistent across studies and sensitivity analyses. In the one study which measured it, there was insufficient evidence of lower 1-Naphthol and 2-Naphthol levels in the HTP group compared with the smoking group.

For NNAL and COHb, certainty of evidence using GRADE considerations (risk of bias, inconsistency, imprecision, indirectness and publication bias) was reported. For NNAL, certainty of the evidence was moderate for the comparisons of HTP versus smoking and abstinence, and for COHb, certainty was moderate for the comparison of HTP versus smoking and very low for the comparison of HTP versus abstinence (1).

Table 4. Comparison of biomarkers of exposure (1)

Outcome	HTP compared with smoking		HTP compared with abstinence	
	Relative effect (95% CI)	Number of participants (studies)	Relative effect (95% CI)	Number of participants (studies)
NNAL	LMD -0.81 (-0.55 to -1.07)	1959 (10)	LMD 0.50 (0.34 to 0.66)	382 (5)
COHb	LMD -0.74 (-0.52 to -0.92)	1807 (9)	LMD 0.69 (0.04 to 1.34) intention-to-treat LMD -0.32 (-1.04 to 0.39) per-protocol	212 (3) intention-to-treat; 170 (2) per-protocol
1-OHP	LMD 0.42 (-0.67 to -0.17)	1,960 (10)	LMD 0.12 (-0.03 to 0.28)	382 (5)
3-HPMA	LMD -0.40 (-0.62 to -0.17)	1,960 (10)	LMD 0.56 (0.33 to 0.80)	382 (5)
MHBMA	LMD -1.15 (-1.52 to -0.78)	1,960 (10)	LMD 0.67 (-0.12 to 1.45)	382 (5)
Exhaled CO	SMD -0.56 (-0.68 to -0.45)	1,322 (3)	Not reported	
1-naphthol	SMD 0.07 (−0.43 to 0.56)	63 (1)	Not reported	
2-naphthol	SMD −0.50 (−1.00 to 0.00)	63 (1)	Not reported	

Notes: CI: Confidence interval; LMD: Difference in means of log-transformed measurements; SMD: standardised mean difference.

Biomarkers of harm

Pooled data from 5 studies with at least 4-week follow-up showed greater lung function measured by FEV1 among those using HTP compared with cigarette smoking and insufficient evidence of a difference between HTP and abstinence groups. The pooled results did not show differences in lung function measured using FVC. There was insufficient evidence for differences in systolic or diastolic blood pressure when HTP use was compared with smoking. There was also insufficient evidence of clinically significant differences in systolic and diastolic blood pressure between HTP and abstinence groups (table 5). There was no substantial heterogeneity among studies that reported on biomarkers of harm (0% to 38%), and no studies reported on measures of FEV1/FVC, heart rate, or blood oxygen saturation (COHb) (1).

Table 5. Comparison of biomarkers of harm/surrogate endpoints (1)

Outcome	HTP compared with smoking		HTP compared with abstinence	
	Relative effect (95% CI)	Number of participants (studies)	Relative effect (95% CI)	Number of participants (studies)
FEV1	LMD 0.02 (0.00 to 0.03)	1,290 (5)	LMD -0.00 (-0.06 to 0.06)	170 (2)
FVC	MD -0.12 (-0.45 to 0.21)	196 (2)	MD -0.02 (-0.29 to 0.26)	172 (2)
Systolic blood pressure	LMD 0.00 (-0.02 to 0.02)	288 (3)	LMD 0.02 (-0.01 to 0.05)	170 (2)
Diastolic blood pressure	LMD 0.00 (-0.03 to 0.03)	288 (3)	LMD 0.00 (-0.04 to 0.04)	170 (2)

Notes: CI: Confidence interval; LMD: Difference in means of log-transformed measurements; MD: Mean Difference.

Adverse events and serious adverse events

Pooled data showed insufficient evidence of a difference in the number of participants reporting adverse events or serious adverse events between those in the HTP use and cigarette smoking groups (table 6). Comparing 5 RCTs that explored differences between HTP and abstinence groups, there was insufficient evidence of a difference in the number of participants in 2 trials reporting adverse events and all 5 studies that reported on serious adverse events reported that none had occurred in either group (table 6). The certainty of the evidence was judged low for the comparison of adverse events in HTP use versus smoking and very low for all other comparisons of adverse or serious adverse events (1).

Table 6. Comparison of adverse and serious adverse events (1)

Outcome	HTP compared with smoking			HTP compared with abstinence		
	Relative effect (95% CI)	Number of participants (studies)	Anticipated absolute effects* (95% CI)	Relative effect (95% CI)	Number of participants (studies)	Anticipated absolute effects (95% CI)*
Adverse events	RR 1.03 (0.92 to 1.15)	1713 (6 RCTs)	Smoking: 235 per 1,000 HTP: 242 per 1,000 (216 to 270)	RR 1.12 (0.86 to 1.46)	237 (2 RCTs)	Abstinence: 468 per 1,000 HTP: 525 per 1,000 (403 to 684)
Serious adverse events	RR 0.79 (0.33 to 1.94)	2009 (9 RCTs)	Smoking: 13 per 1,000 HTP: 10 per 1,000 (4 to 24)	No serious adverse events reported	533 (5 RCTs)	-

Notes: CI: Confidence interval; RCT: Randomised Controlled Trial; RR: Risk ratio

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

Smoking prevalence

Smoking prevalence was not assessed, but 2 studies assessed changes in cigarette sales in Japan in relation to HTP use. One study found that the yearly percentage decline in cigarette sales accelerated after the introduction of HTP, increasing from an average decline of -3.1% across 2011 to 2015 to -16.4% across 2016 to 2019 (14). The second study found that per capita cigarette sales were increasing at a rate of 0.10 to 0.14 (depending on statistical approach) per month before the introduction of HTP and declined at a rate of 0.63 to 0.66 cigarettes per month after the introduction of HTP (15). The certainty of the evidence was judged to be very low (1).

14.5 Conclusions

Use of HTP in England

Among young people aged 11 to 18 in the ASH-Youth survey, 0.9% had tried but no longer used and 0.3% reported currently using HTP.

Among young people aged 16 to 19 in the ITC Youth survey, 1.5% had ever tried HTP but not used them in the past week and 0.7% had used HTP in the past week.

Two thirds (65.7%) of young people aged 16 to 19 who had ever tried HTP had used it once or up to 10 times only.

Among adults in England, 0.3% in the STS and 0.5% in ASH-Adult survey reported currently using HTP.

The proportion of adults who reported having ever used HTP was 1.8%. It was more common among people aged 25 to 34, women, and adults who smoked or vaped.

One third of ever or current adult users of HTP had tried HTP once or twice and 16% of current users (less than 0.1% of adults in England) reported daily use.

Among past year smokers who had attempted to stop smoking, 1.6% reported having used HTP to support their attempt.

Cochrane review

The Cochrane review of HTP for smoking cessation and reducing smoking prevalence reported no studies reported on HTP use for cigarette smoking cessation, so the effectiveness of HTP for stopping smoking remains uncertain.

The Cochrane review found moderate certainty evidence that smokers switching to HTP use have lower exposure to toxicants and carcinogens than smokers continuing to smoke. There was moderate- to very low-certainty evidence of higher exposure compared with those attempting abstinence from all tobacco.

There was some evidence for people improving the amount of air they can exhale from the lungs (FEV1) after switching to HTP use compared with continuing to smoke, but there was insufficient evidence of difference for other biomarkers of harm.

There was insufficient evidence for differences in risk of adverse or serious adverse events between people randomised to switch to HTP, smoke cigarettes or attempt tobacco abstinence in the short-term.

The rate of decline in cigarette sales accelerated after the introduction of HTP to market in Japan. However, it is possible that other factors caused this change. A decline in cigarette sales may not translate to declining smoking prevalence, and changes in Japan may not generalise elsewhere.

14.6 Implications

Monitoring of uptake among young people and adults should continue.

Independently funded research is needed into whether HTP helps people stop smoking, their safety, and the impact of HTP use on smoking rates.

14.7 References

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15 Harm perceptions and communications

15.1 Introduction

The objective of this chapter is to summarise the evidence on harm perceptions of vaping products among the general public, including what influences these harm perceptions and the influence perceptions have on vaping and smoking behaviours. Harm perceptions include perceptions of the relative harms of vaping products and cigarettes, perceptions of absolute harms of vaping products (that is, not relative to cigarettes) and perceived addictiveness, where these were assessed.

As discussed in previous chapters, our assessment of the evidence is that vaping products are substantially less harmful to overall health than smoking. Perceptions of relative harm of vaping products and cigarettes were therefore viewed as accurate if vaping products were perceived as overall lower harm than cigarettes. Perceptions of relative harm of vaping products and cigarettes were viewed as inaccurate if indicating overall equal, greater, or unknown harm from vaping products relative to cigarettes.

We summarise evidence from surveys among young people and adults to assess these harm perceptions, and, where possible, we also assessed changes over time. We also include data from a systematic review of peer reviewed literature published between January 2007 and June 2021 examining 2 research questions: what interventions have been effective in changing vaping harm perceptions; and, to what extent are vaping harm perceptions predictive of any changes in vaping and smoking behaviours?

15.2 Methods

In line with chapter 3 on vaping among young people, we have drawn on 2 online surveys carried out in recent years, predominantly the ASH-Y survey (covering 11 to 18 year olds), supplemented where appropriate with data from the International Tobacco Control Policy Evaluation Project (ITC) Youth Tobacco and Vaping survey (covering 16 to 19 year olds). The methods for these surveys are given in chapter 2. Similarly, in line with chapter 4 on vaping among adults, we have drawn on the Smoking Toolkit Survey (age 18+ years), and the ASH-A survey (age 18+ years). The methods for these surveys are also given in chapter 2. The systematic review methods are also given in chapter 2.

15.3 Harm perceptions among young people

Harm perceptions of vaping relative to smoking (ASH Youth and ITC Youth)

As discussed in previous chapters, our assessment of the evidence is that vaping products are substantially less harmful to overall health than smoking. Among 11 to 18 year olds (ASH-Y) in 2021, 44.7% said they thought that vaping products were less harmful than smoking, indicating that most youth (55.3%) did not know the correct answer. Around a third (32.4%) inaccurately thought that the harms from vaping and smoking were about the same, with an additional 3.6% inaccurately thinking that vaping was more harmful than smoking. Many young people, however, were uncertain, with 19.3% of 11 to 18 year olds saying they did not know which was more harmful. These ‘don’t know’ responses were more common among younger ages, with 25.5% of 11 to 15 year olds reporting they did not know the relative harms of vaping compared to smoking compared to 10.9% of 16 to 17 year olds and 10.8% of 18 year olds (table 1).

ASH-Y data suggest that young people’s perceptions of the relative harms from vaping and smoking have changed since 2015 (Figure 1) with the proportion who accurately thought that vaping was less harmful than smoking declining from 66.7% in 2015 to 43.3% in 2020, and then increasing slightly in the past year to 44.7%. Over the same time period, the proportion of young people who inaccurately thought that the harms were the same increased from 21.2% in 2015 to 35.3% in 2020, decreasing slightly to 32.4% in 2021. The proportion of young people who inaccurately thought that vaping was more harmful than smoking has been low throughout, whereas the proportion not knowing has increased from 9.9% in 2015 to 19.3% in 2021 (Figure 1).

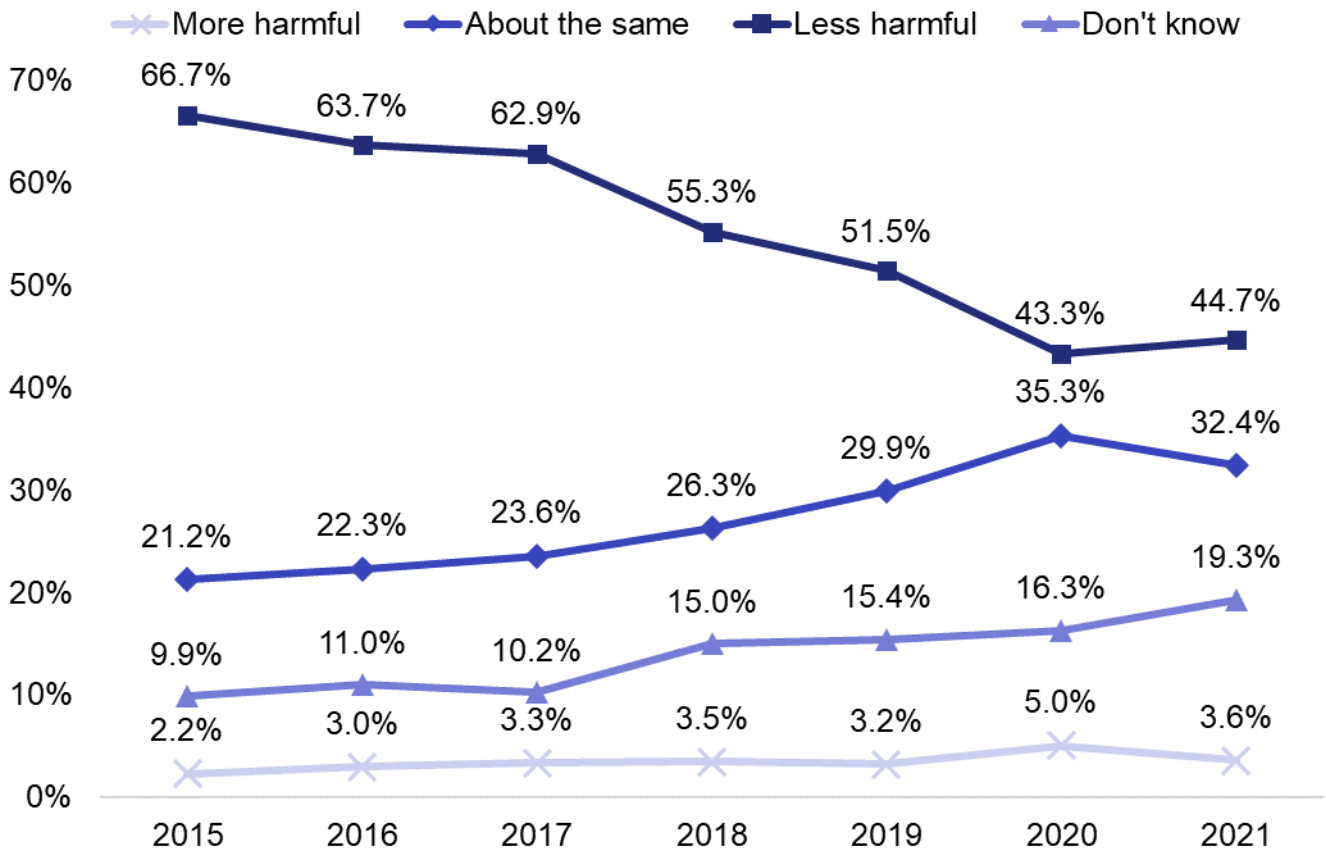
Inaccurate perceptions that vaping is more or equally as harmful as smoking were similar between those who currently vaped (36.4%) and those who never vaped (33.3%). However, the majority of youth who currently vaped accurately perceived vaping as less harmful than cigarettes (62.1%), (table 1). For current smokers, 39.3% thought vaping was more or equally as harmful as smoking, with 50.0% accurately perceiving vaping as less harmful than smoking. Never vapers and never smokers had a high proportion of don’t know responses (21.4% and 21.1%, respectively) (table 1).

Table 1. Perceptions of the relative harms of vaping and smoking among 11 to 18 year olds, by age, gender, region, social grade and smoking status, England 2021 (ASH-Y, weighted data)

	More harmful % (n)	About the same % (n)	Less harmful % (n)	Don't know % (n)
Total	3.6 (70)	32.4 (637)	44.7 (879)	19.3 (379)
Age				
11 to 15	3.7 (45)	31.7 (384)	40.0 (485)	24.5 (297)
16 to 17	4.0 (20)	34.0 (168)	51.0 (252)	10.9 (54)
18	1.9 (5)	32.7 (85)	54.6 (142)	10.8 (28)
Gender				
Female	3.3 (33)	29.4 (293)	49.2 (491)	18.1 (181)
Male	3.8 (37)	35.6 (345)	40.1 (388)	20.5 (198)
Region				
North	3.1 (17)	30.7 (170)	47.3 (262)	19.0 (105)
Midlands	3.1 (12)	34.0 (133)	39.6 (155)	23.3 (91)
South	4.0 (41)	32.8 (335)	45.2 (462)	18.0 (184)
Social grade				
ABC1	3.6 (50)	31.3 (440)	46.8 (658)	18.4 (259)
C2DE	3.6 (20)	35.4 (198)	39.5 (221)	21.5 (120)
Smoking status				
Never smoker	3.1 (50)	32.4 (529)	43.5 (710)	21.1 (345)
Tried only	5.1 (9)	32.2 (57)	53.7 (95)	9.0 (16)
Former smoker	10.6 (6)	30.5 (18)	49.2 (29)	10.2 (6)
Current smoker	4.8 (4)	34.5 (29)	50.0 (42)	10.7 (9)
Vaping status				
Never vaper	3.0 (50)	33.4 (551)	42.2 (696)	21.4 (353)
Tried only	3.7 (7)	29.1 (55)	58.2 (110)	9.0 (17)
Former vaper	19.2 (5)	26.9 (7)	53.8 (14)	0
Current vaper	5.7 (5)	27.6 (24)	62.1 (54)	4.6 (4)

Notes: Unweighted base = 1,944. Never smokers were people who had never tried cigarettes. Tried only smokers were people who had only ever tried smoking cigarettes once. Former smokers were people who used to smoke sometimes but who never smoked now. Current smokers were people who smoked sometimes but less than weekly, as well as those who smoked more than once a week.

Figure 1. Perceptions of the relative harms of vaping compared with smoking among 11 to 18 year olds, by year, England, 2015 to 2021 (ASH-Y weighted data)

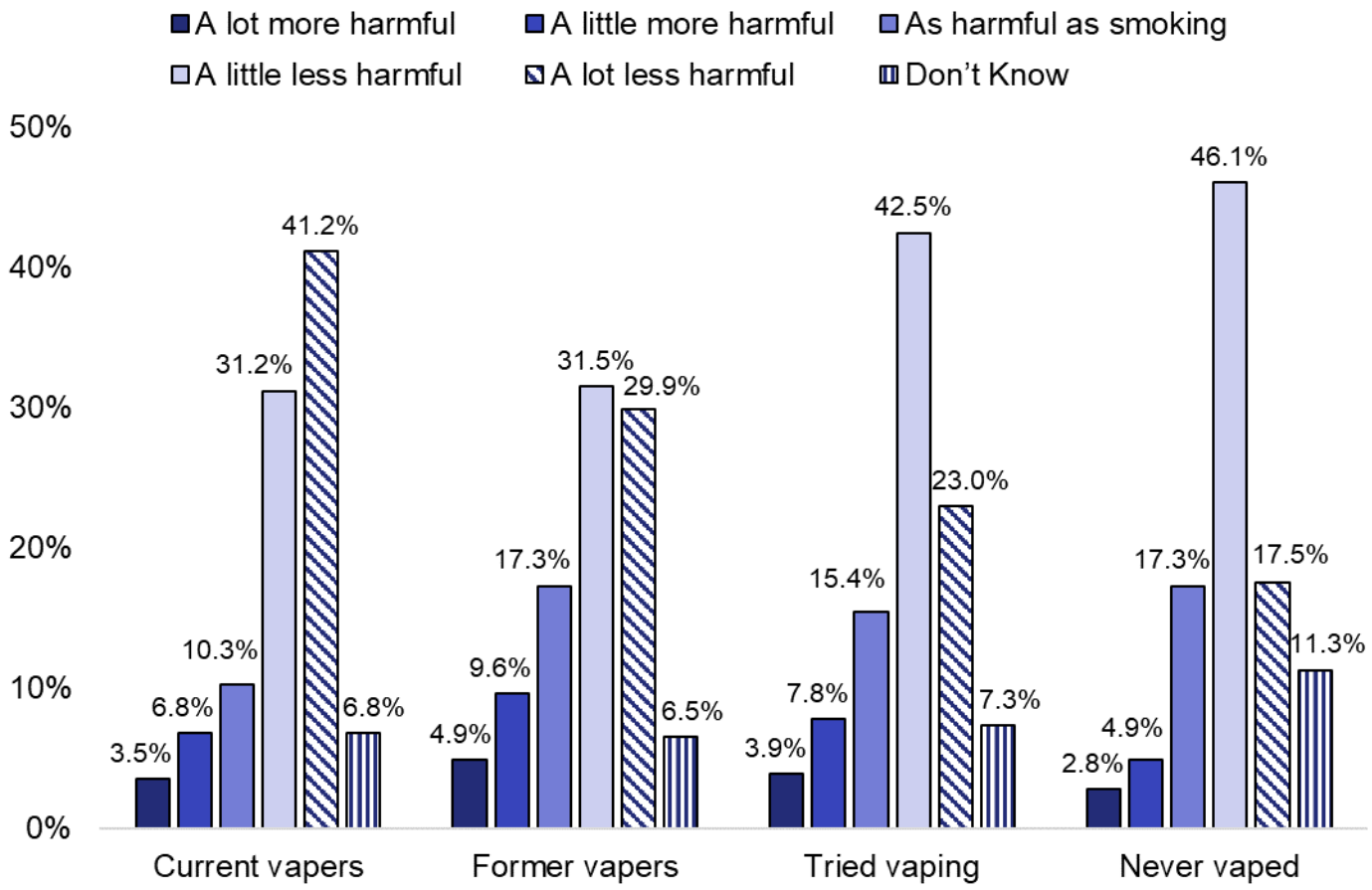


Notes: Unweighted bases; 2015=1,797; 2016=1,859; 2017=2,077; 2018=1,878; 2019=2,057; 2020=2,031; 2021=1,944.

Data from the ITC Youth (16 to 19 years old) survey showed slightly different patterns from ASH-Y data in 2021, with the majority (62.9%) accurately perceiving vaping as less harmful than smoking, 16.8% inaccurately perceiving vaping to be equally harmful, 10.0% inaccurately perceiving vaping to be more harmful than smoking and 10.0% reporting that they didn't know. Similar to the ASH-Y findings, ITC reported broadly similar levels of inaccurately perceiving vaping to be equally or more harmful than smoking among never vapers (25.0%) as current vapers (20.6%), and slightly higher levels of accurately perceiving vaping to be less harmful than smoking among current vapers (72.4%) than never vapers (63.6%) (Figure 2).

Figure 2. Perceptions of the relative harms of vaping compared with smoking among 16 to 19 year olds, by vaping status, England 2021 (ITC weighted data)

ITC 2021 aged 16 to 19



Notes: Unweighted base = 4,298.

Never vapers were people who had never tried vaping. Tried only vapers were people who had tried vaping, but who had vaped on no more than 10 days in their life. Former vapers were people who had vaped on more than 10 days in their life, but who had not vaped in the past 30 days. Current vapers were people who had vaped on more than 10 days in their life and who had vaped in the past 30 days.

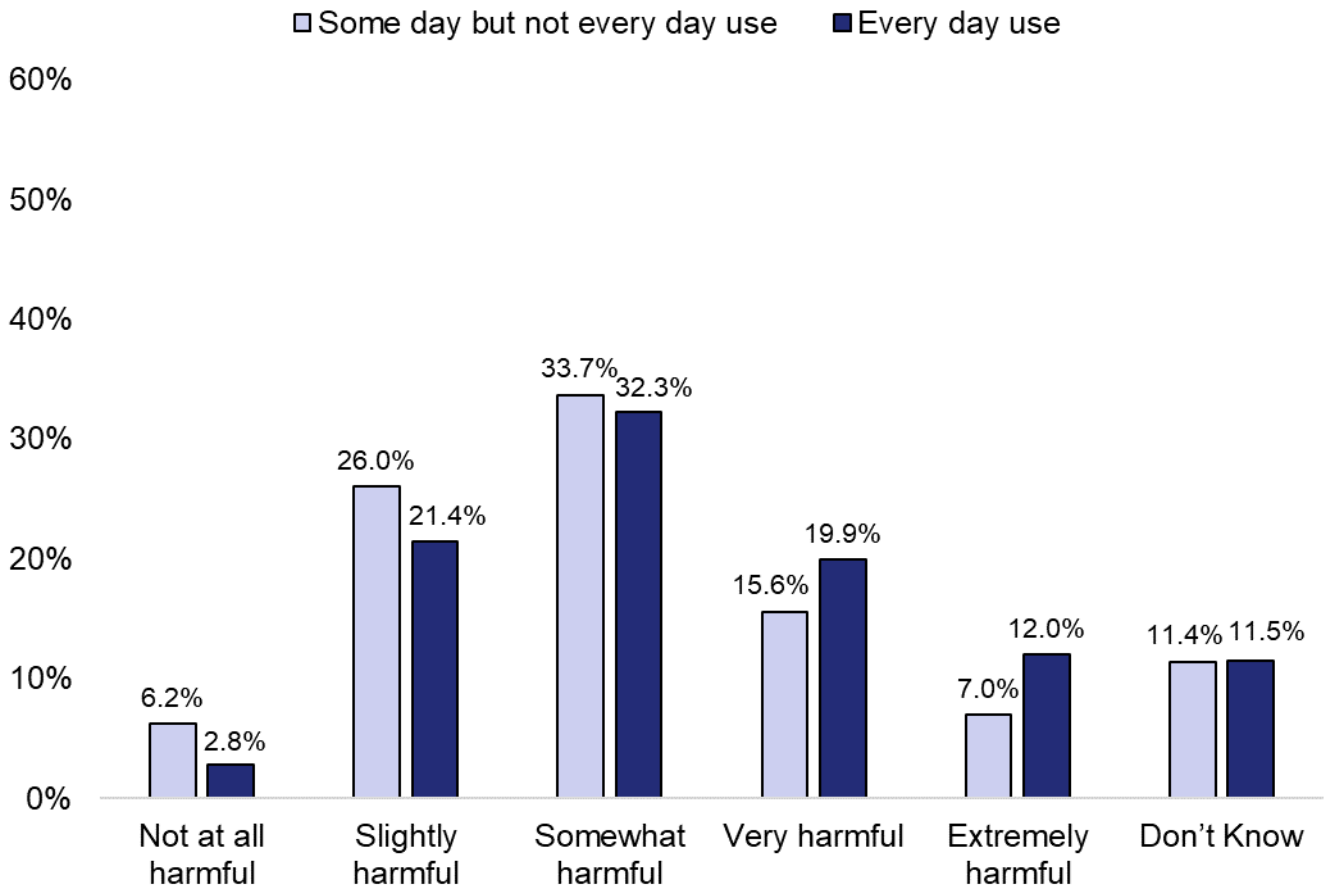
Absolute harm perceptions of vaping and smoking (ITC Youth)

The ITC Youth survey also asked all 16 to 19 year olds how harmful it was to vape or smoke every day or some day but not every day. When asked about vaping, respondents reported broadly similar levels of harm perceptions for 'some day but not every day' and 'every day' use, but did show indications of a dose-response effect with "slightly harmful" being more common for some day use than every day use (26% vs. 21.4%) and "very" and "extremely harmful' being 4.3 to 5 percentage points higher for every day use compared

with some day use (Figure 3). Around half of respondents viewed daily (53.7%) and some day (59.7%) vaping as “slightly” or “somewhat” harmful. Slightly fewer respondents viewed occasional vaping (22.6%) as “very” or “extremely” harmful, compared to daily vaping (31.9%). Around one in ten did not know the harms of some day (11.4%) or daily (11.5%) vaping, and few participants perceived occasional (6.2%) or daily (2.8%) vaping as “not at all” harmful (Figure 3).

Figure 3. Perceptions of harm from every day, and some days but not every day, vaping among 16 to 19 year olds; England 2021 (ITC, weighted data)

ITC aged 16 to 19



Notes: Unweighted base n= 4,298.

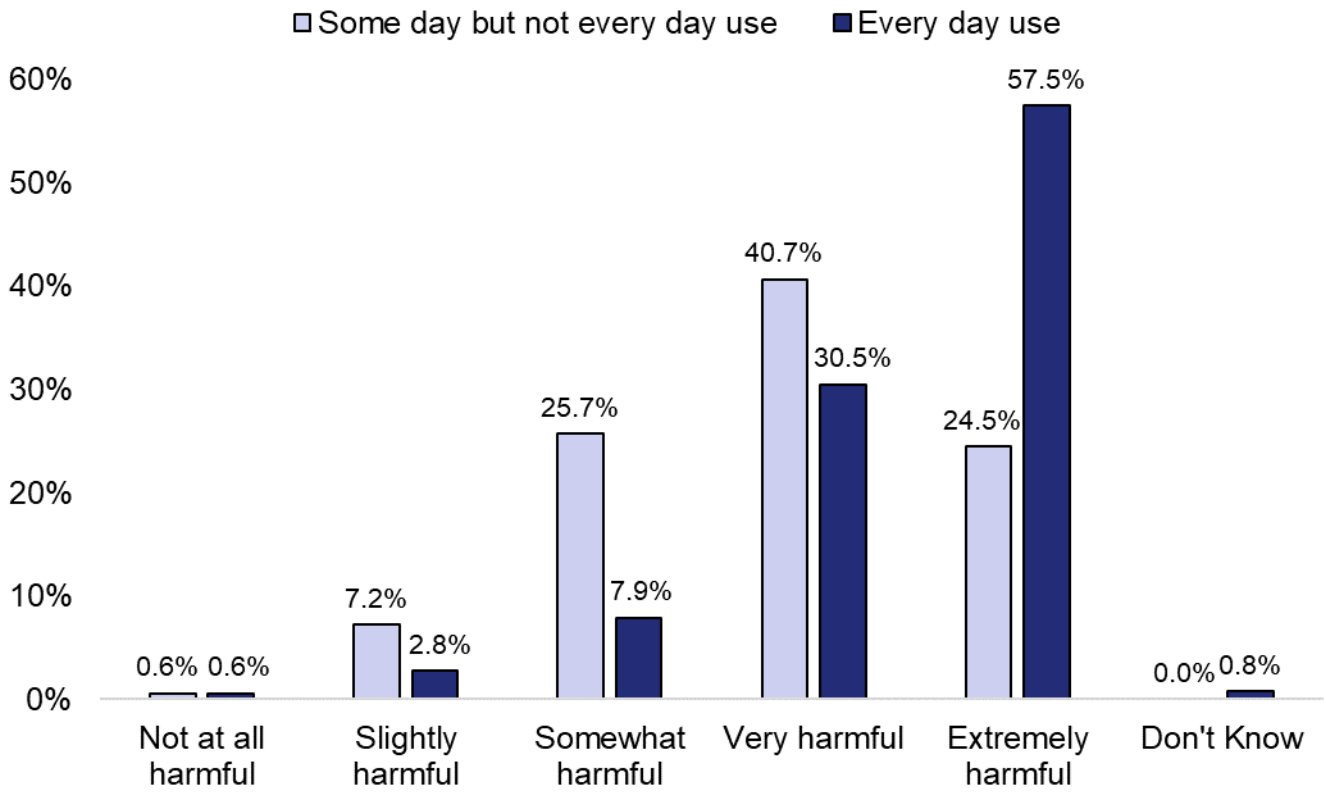
In contrast, a majority of respondents rated smoking cigarettes as more harmful, with some day but not every day smoking (65.2%) and every day smoking (88.0%) rated as “very” or “extremely” harmful, compared with a minority of respondents rating some day vaping and every day vaping as “very” or “extremely” harmful (22.6% and 31.9%, respectively). One-third of 16 to 19 year olds perceived some day smoking to be “slightly” or “somewhat” harmful (32.9%), while 10.7% perceived daily smoking to be slightly or

somewhat harmful (compared with 53.7% perceiving daily vaping to be slightly or somewhat harmful). Few 16 to 19 year olds reported occasional (0.6%) or daily smoking (0.6%) as “not at all” harmful, and there were also very few “don’t know” responses (0.8% daily, 0.0% some day) (Figure 4).

In summary, 16 to 19 year olds rated daily smoking higher on the scale of harm than they rated some day use, with daily smoking seen as over twice as likely to be extremely harmful as some day smoking (57.5% vs. 24.5%). For daily vaping, only 12% rated it as extremely harmful and there was less difference in vaping perceptions between some day and daily use. Greater proportions of 16 to 19 year olds perceived some day or daily vaping as not at all harmful than smoking; however, proportions for both were still very small. A greater proportion of 16 to 19 year olds did not know the harms of vaping than did not know the harms of smoking.

Figure 4. Perceptions of harm from every day, and some days but not every day, cigarette smoking among 16 to 19 year olds; England 2021 (ITC, weighted data)

ITC aged 16 to 19



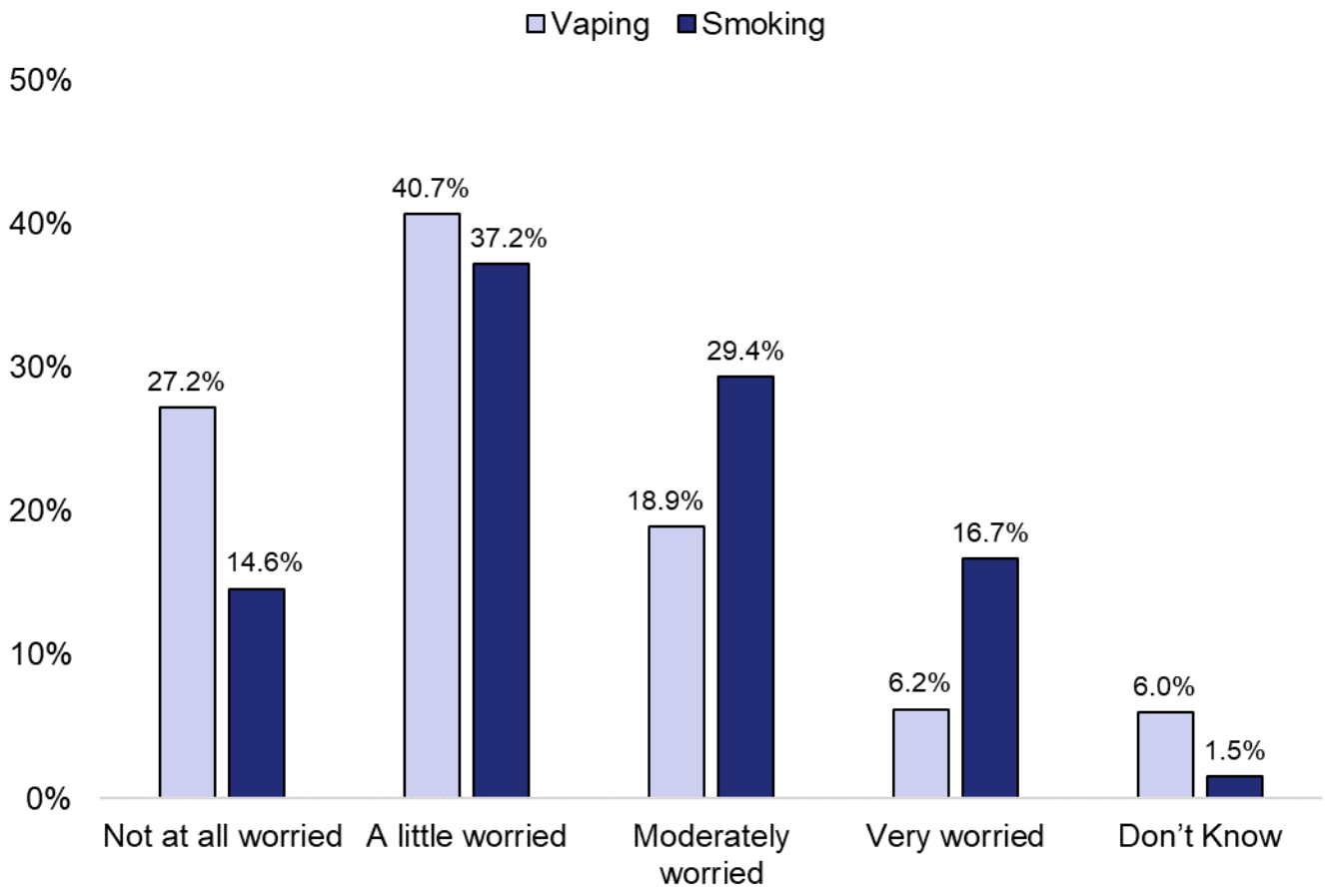
Notes: Unweighted base n= 4,298.

Among past 30-day vapers, 40.7% were ‘a little’ worried that vaping would damage their health in the future, and 27.2% were ‘not at all’ worried, while one-quarter (25.1%) reported being ‘moderately’ or ‘very’ worried. Few (6.0%) reported ‘don’t know’ (Figure 5).

In contrast, among past 30-day smokers, most (46.1%) 16 to 19 year olds were ‘moderately’ or ‘very’ worried that smoking would damage their health in the future, 37.2% were ‘a little’ worried, and 14.6% were ‘not at all’ worried. Very few (1.5%) reported ‘don’t know’ (Figure 5).

Figure 5. Worry about vaping, and cigarette smoking, harm to future health among past 30-day vapers and smokers among 16 to 19 year olds; England 2021 (ITC, weighted data)

ITC aged 16 to 19



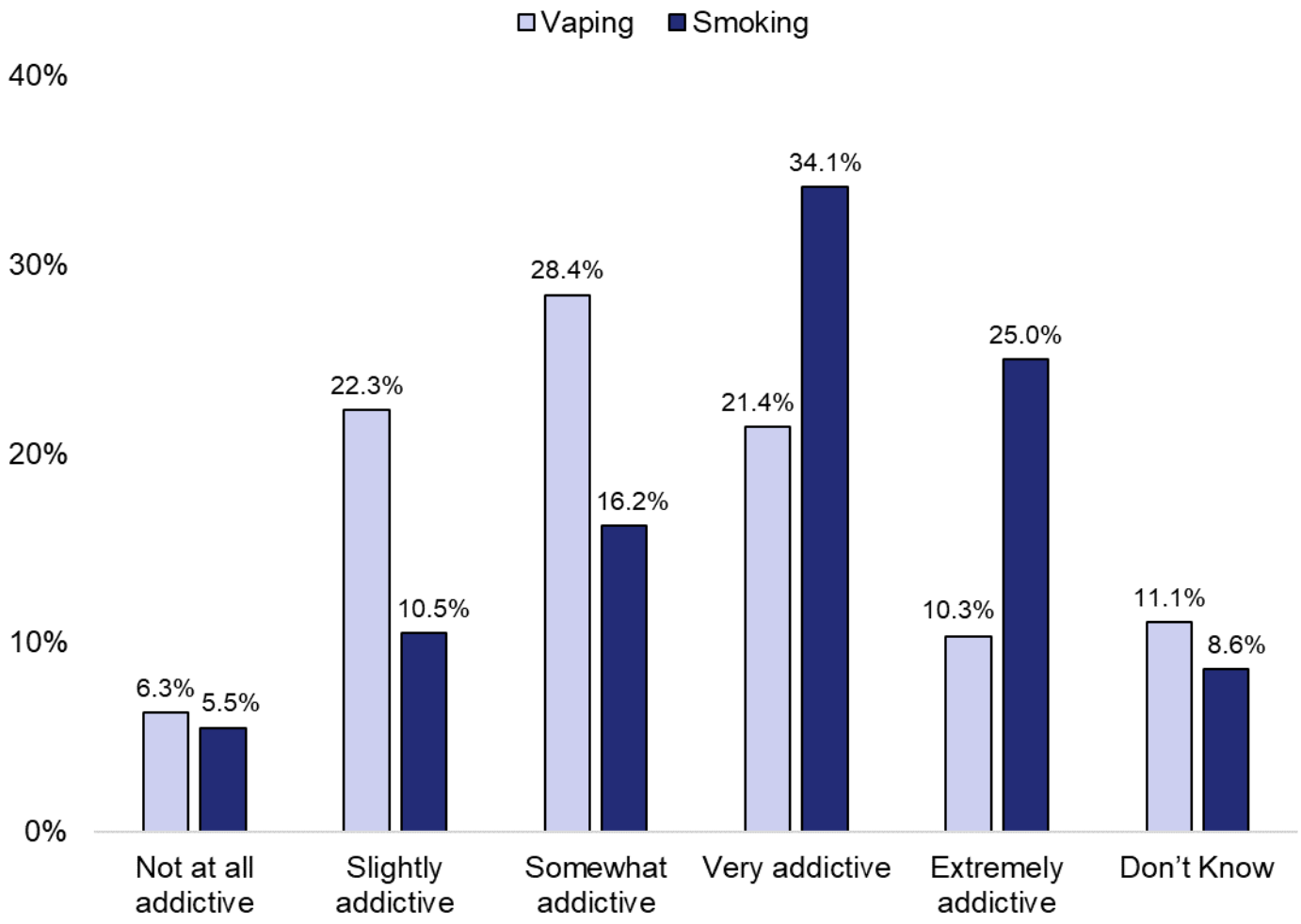
Notes: Unweighted base; vapers n=567, smokers n= 656.

Perceived addictiveness of vaping and smoking (ITC Youth)

In the ITC Youth survey, 16 to 19 year olds were asked ‘In your opinion, how addictive are e-cigarettes/vaping?’ Half perceived vaping to be ‘slightly’ or ‘somewhat’ addictive (50.7%), one-third perceived vaping to be ‘very’ or ‘extremely’ addictive (31.7%), and few (6.3%) perceived e-cigarettes to be ‘not at all’ addictive; 11.1% did not know (Figure 6).

When asked about smoking, over half perceived cigarettes to be ‘very’ or ‘extremely’ addictive (59.1%), followed by ‘slightly’ or ‘somewhat’ addictive (26.7%); 5.5% perceived them as not at all addictive. The remainder (8.6%) did not know how addictive cigarettes were (Figure 6).

Figure 6. Perceptions of the addictiveness of vaping, and cigarette smoking, among 16 to 19 year olds; England 2021 (ITC, weighted data)



Notes: Unweighted base N=4,298.

Perceptions of vaping as a smoking cessation aid

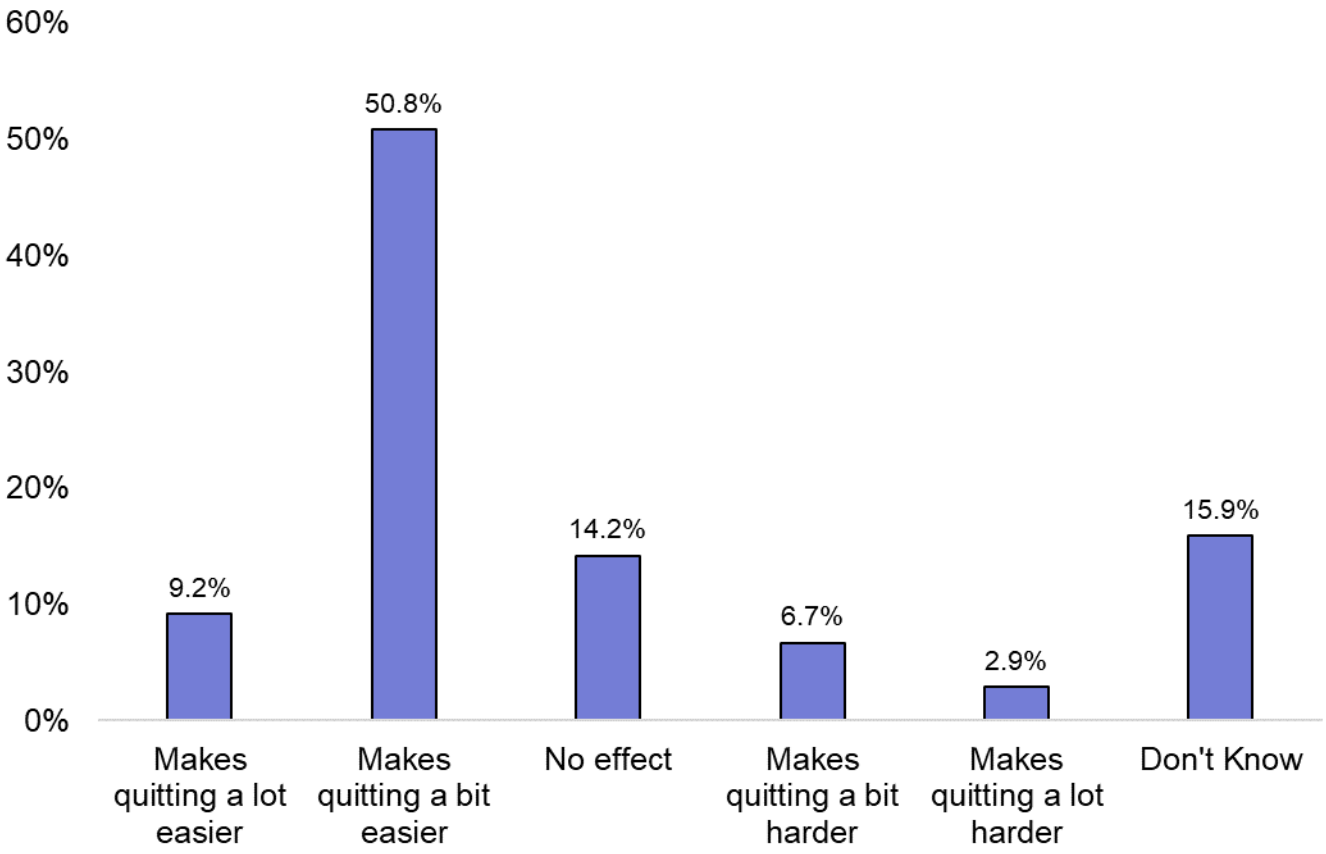
When 16 to 19 year olds were asked if they thought vaping made it easier or harder to permanently quit smoking cigarettes, over half perceived that vaping makes quitting smoking ‘a bit’ or ‘a lot easier’ (60.0%), many (14.2%) thought it had ‘no effect’, just under one-tenth (9.6%) perceived that vaping made quitting ‘a bit’ or ‘a lot harder’, with 15.9% saying that they did not know (Figure 7).

When investigated by smoking and vaping status, most current vapers (67.7%) and former smokers (67.1%) perceived that vaping made quitting smoking easier, whereas 58.3% of current smokers perceived that vaping made quitting easier. Almost a quarter of current smokers perceived vaping had no effect on quitting (22.3%), while fewer current vapers (13.2%) perceived no effect (table 2).

Nearly one-quarter of 16 (22.7%) and 17 (22.6%) year olds responded ‘don’t know’, compared with 11.2% of 18 and 12.6% of 19 year olds (table 2).

Figure 7. Perceived effect of vaping on permanently quitting smoking among 16 to 19 year olds; England 2021 (ITC, weighted data)

ITC aged 16 to 19



Notes: Unweighted base N=4,298.

Table 2. Perceptions of vaping to help quit smoking among 16 to 19 year olds, overall and by sociodemographic and smoking and vaping status; England 2021 (ITC, weighted data)

	Makes quitting a lot or a bit easier % (n)	No effect %(n)	Makes quitting a lot or a bit harder % (n)	Don't know %(n)
Overall	60.0 (2580)	14.2 (611)	9.6 (414)	16.0 (688)
Age				
16	56.0 (518)	13.6 (126)	7.8 (72)	22.7 (207)
17	57.7 (680)	14.4 (170)	8.6 (101)	19.2 (226)
18	64.5 (971)	13.9 (210)	10.3 (155)	11.2 (168)
19	59.7 (411)	15.2 (105)	12.5 (86)	12.6 (87)
Gender				
Female	57.5 (1270)	15.3 (339)	9.3 (206)	17.9 (395)
Male	62.7 (1309)	13.0 (272)	10.0 (208)	14.0 (292)
Region				
North	63.3 (763)	13.9 (168)	7.8 (94)	14.8 (179)
Midlands	60.5 (520)	14.4 (124)	9.8 (84)	15.1 (130)
South	58.1 (1296)	14.3 (319)	10.5 (235)	17.0 (379)
Ethnicity				
White	61.4 (1891)	14.5 (446)	8.2 (252)	15.8 (488)
Black and minority ethnic groups	56.4 (658)	13.7 (160)	13.5 (158)	16.1 (188)
Smoking status				
Never smoker	58.6 (1459)	13.1 (327)	9.9 (247)	18.3 (455)
Tried only	62.6 (862)	14.3 (197)	9.0 (124)	13.8 (190)
Former smoker	67.1 (49)	15.1 (11)	11.0 (8)	6.8 (5)
Current smoker	58.3 (196)	22.3 (75)	10.4 (35)	8.9 (30)
Vaping status				
Never vaper	56.0 (1382)	14.3 (352)	10.0 (248)	19.7 (486)
Tried only	65.9 (705)	13.6 (146)	8.7 (93)	11.5 (123)
Former vaper	61.7 (227)	16.8 (62)	10.9 (40)	10.6 (39)
Current vaper	67.7 (266)	13.2 (52)	8.4 (33)	10.2 (40)

Notes: Unweighted base = 4,298.

Never smokers were people who had never tried cigarettes. Tried only smokers (referred to as 'Experimental smokers' in the ITC survey) were people who had tried cigarettes, but who had not smoked more than 100 cigarettes in their life. Former smokers were people who had smoked more than 100 cigarettes in their life, but who had not smoked in the past 30 days. Current smokers were people who had smoked more than 100 cigarettes in their life and who had smoked in the past 30 days. Never vapers were people who had never tried vaping. Tried only vapers were people who had tried vaping, but who had vaped on no more than 10 days in their life. Former vapers were people who had vaped on more than 10 days in their life, but who had not vaped in the past 30 days. Current vapers were

people who had vaped on more than 10 days in their life and who had vaped in the past 30 days.

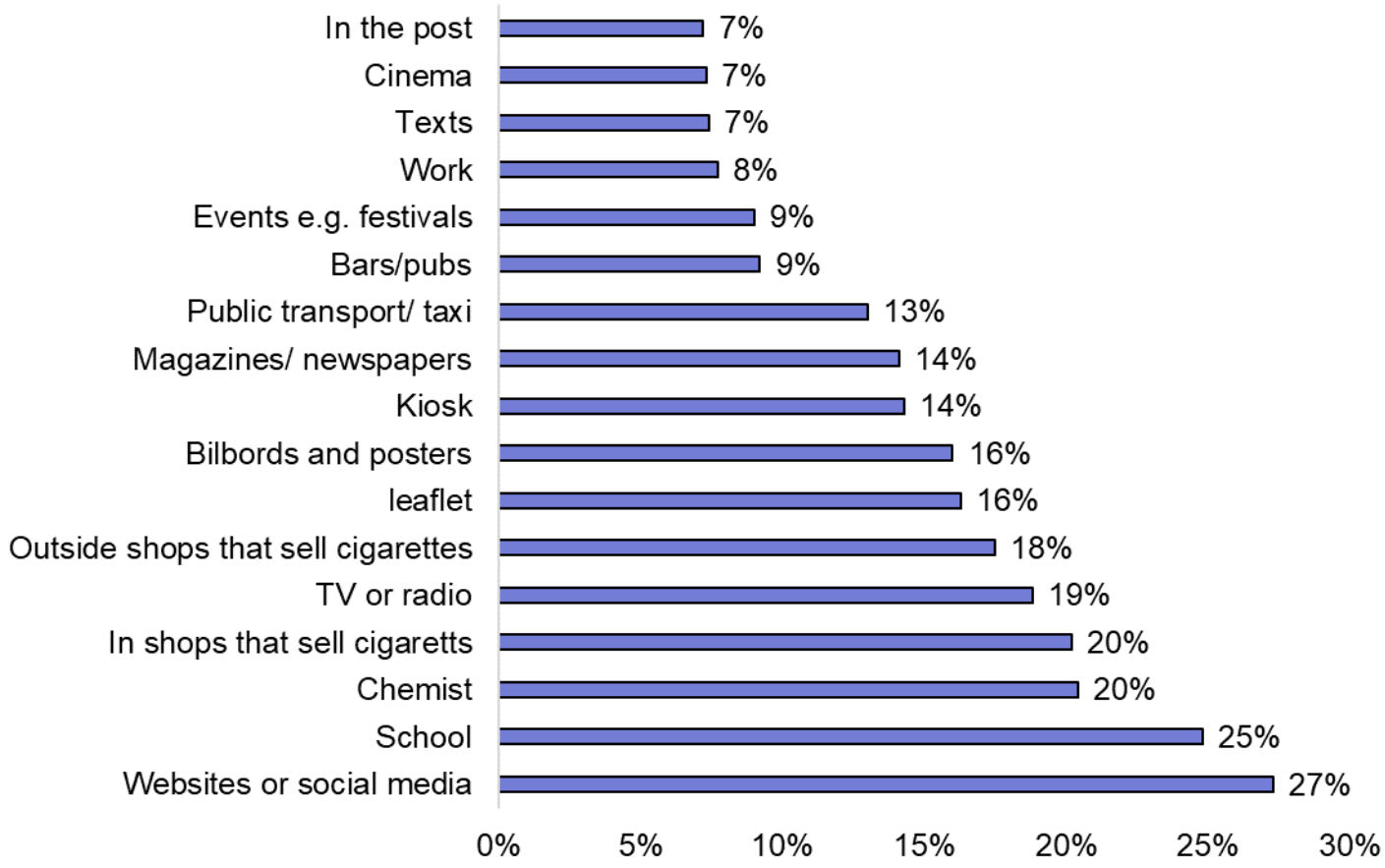
Those who reported they did not know their ethnicity (n=51) were excluded from the Ethnicity frequencies.

Exposure to campaigns

In the ITC Youth survey, 16 to 19 year olds were asked if they had noticed any education campaigns or public health messages about vaping in each of 18 places, in the past 12 months. Overall, just over half reported noticing any education campaign or public health message about vaping in the past 12 months (53.0%). Educational campaigns or public health messaging were most commonly noticed on social media (27.3%), at school (24.8%), at chemists (20.4%), or in shops that sell cigarettes (20.2%) (Figure 8).

Noticing any educational or public health campaigns was least common among those who had never vaped (49.3%), with 58.2% of those who had ever vaped having noticed a campaign. Likewise, noticing was least common among those who had never smoked (49.8%), and 57.3% of those who had ever smoked had noticed a campaign. Noticing appeared to increase with age. Among those from a black or ethnic minority background, (61.1%) reported noticing compared with 49.9% from a white background. Over half (57.7%) of females reported noticing campaigns compared with 48.5% of males (table 3).

Figure 8. Locations for noticing educational campaigns about vaping in the past 12 months among 16 to 19 year olds, England 2021 (ITC, weighted data)



Notes: Unweighted base: n=4,298.

Table 3. Noticing educational campaigns about vaping in the past 12 months among 16 to 19 year olds, overall and by sociodemographics and smoking and vaping status; England 2021 (ITC, weighted data)

	Noticed any educational campaign	Noticed on social media	Noticed at school	Noticed at a chemist
Overall	53.0 (2276)	27.3 (1173)	24.8 (1064)	20.4 (877)
Age				
16	47.4 (438)	23.7 (291)	24.2 (224)	16.7 (155)
17	50.0 (589)	23.7 (279)	23.3 (275)	18.8 (222)
18	55.5 (836)	30.8 (464)	26.5 (399)	22.2 (334)
19	60.0 (413)	30.7 (211)	24.1 (166)	24.0 (165)
Gender				
Female	57.7 (1204)	29.8 (623)	25.8 (539)	24.2 (505)
Male	48.5 (1072)	24.9 (550)	23.8 (526)	16.8 (371)
Region				
North	51.6 (623)	26.3 (317)	24.1 (291)	20.0 (241)
Midlands	52.6 (452)	26.3 (226)	22.2 (191)	20.5 (176)
South	53.8 (1201)	28.2 (630)	26.1 (583)	20.6 (460)
Ethnicity				
White	49.9 (1573)	25.3 (781)	23.2 (714)	19.4 (597)
Black and minority ethnic groups	61.1 (713)	32.6 (380)	28.6 (334)	22.9 (267)
Smoking status				
Never smoker	49.8 (1241)	25.5 (635)	23.3 (581)	18.2 (454)
Tried only	57.8 (796)	29.3 (403)	28.2 (389)	23.1 (318)
Former smoker	55.6 (40)	34.7 (25)	29.2 (21)	31.9 (23)
Current smoker	56.3 (189)	31.6 (106)	20.0 (67)	23.5 (79)
Vaping status				
Never vaper	49.3 (1217)	24.6 (339)	23.7 (584)	17.9 (441)
Tried only	58.7 (627)	31.7 (339)	27.5 (294)	24.3 (260)
Former vaper	55.6 (205)	30.9 (114)	25.8 (95)	21.7 (80)
Current vaper	57.7 (226)	28.8 (113)	23.3 (91)	24.3 (95)

Notes: Unweighted base = 4,298.

Never smokers were people who had never tried cigarettes. Tried only smokers (referred to as ‘Experimental smokers’ in the ITC survey) were people who had tried cigarettes, but who had not smoked more than 100 cigarettes in their life. Former smokers were people who had smoked more than 100 cigarettes in their life, but who had not smoked in the past 30 days. Current smokers were people who had smoked more than 100 cigarettes in their

life and who had smoked in the past 30 days. Never vapers were people who had never tried vaping. Tried only vapers were people who had tried vaping, but who had vaped on no more than 10 days in their life. Former vapers were people who had vaped on more than 10 days in their life, but who had not vaped in the past 30 days. Current vapers were people who had vaped on more than 10 days in their life and who had vaped in the past 30 days.

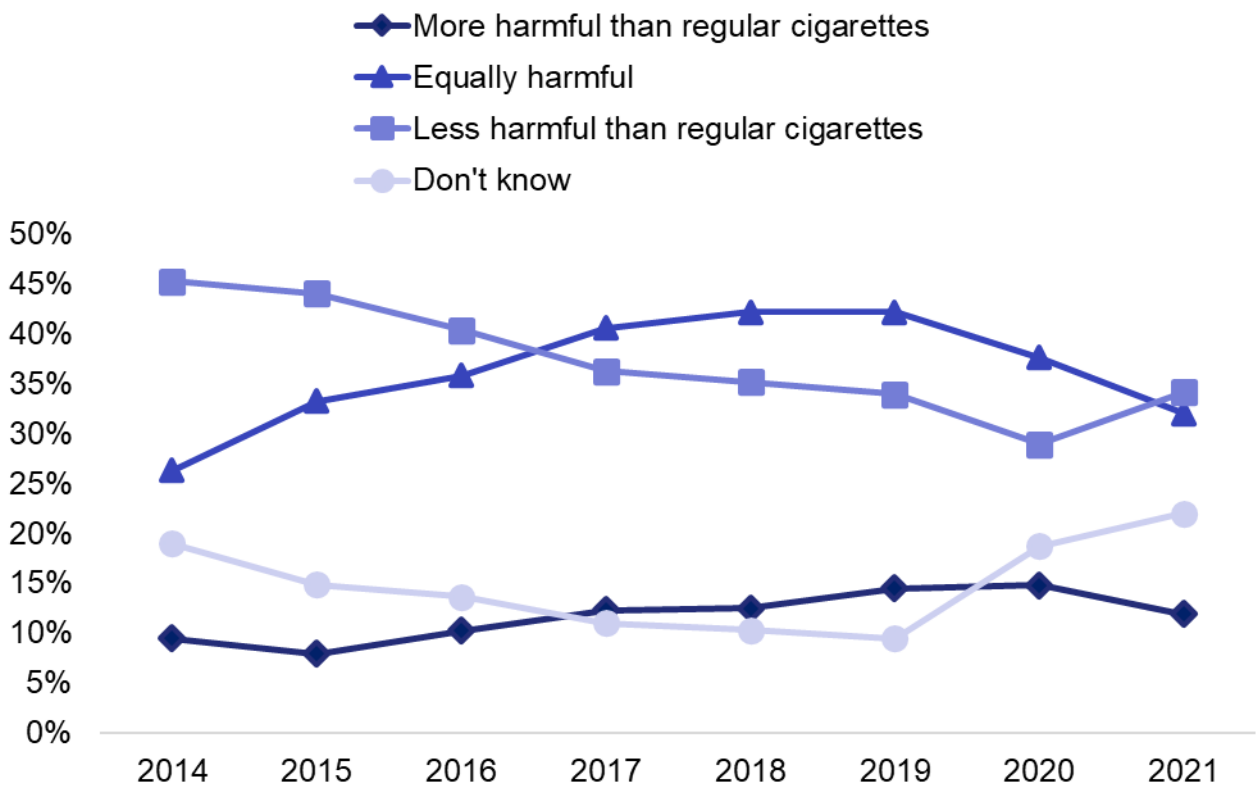
Those who reported they did not know their ethnicity (n=51) were excluded from the Ethnicity frequencies.

15.4 Harm perceptions among adults

Overall among adult smokers, in 2021, 34.1% accurately perceived that vaping was less harmful than smoking, indicating that the majority (65.9%) of adult smokers did not know the correct answer. Similar to patterns among youth, around a third (32.1%) thought that the harms from vaping and smoking were about the same, with 11.9% thinking that vaping was more harmful than smoking and 22.0% said that they did not know which was more harmful. The trend in changes in perceptions about the relative harms of vaping among smokers seem to have changed in the last year (STS – Figure 9). In particular, the proportions of smokers who inaccurately thought that vaping was more harmful or equally harmful than smoking have declined since 2020 by 2.9 and 5.6 percentage points, respectively, and the proportion of smokers who accurately believe that vaping is less harmful than smoking increased by 5.0 percentage points (the first time an increase in this measure had been observed since 2014). In addition, there seems to be growing confusion regarding the relative harms of vaping, as the proportion of smokers who did not know whether smoking or vaping was more harmful has more than doubled from 9.5% in 2019 to 22.0% in 2021. Overall, the pattern indicates that in 2021 the large majority (66.9% or two-thirds) either ‘don’t know’ or mistakenly think the vaping is equally or more harmful than smoking cigarettes. These recent trends are similar to those observed among young people as reported above.

In the 2021 report commissioned by PHE (1), we purported that the decline in smokers believing vaping to be less harmful between 2019 and 2020 and an increase in the proportion of smokers who did not know about the relative harms of vaping, were likely influenced by the e-cigarette or vaping use-associated lung injury (EVALI) outbreak in the US in late 2019 (2).

Figure 9. Harm perceptions about vaping among current smokers, England 2014 to 2021 (STS, weighted data)



Notes: Age 18+. Unweighted bases: 2014=663; 2015=1,223; 2016=3,664; 2017=3,379; 2018=3,523; 2019=3,220; 2020=2,952; 2021 (to September) =3,157. Current smokers included people who said that they smoked daily or that they smoked, but less than daily. 2021 data available from January to September. The full year's data was used for all other years.

The proportion of smokers who did not know whether vaping or smoking was more harmful appeared to increase with age and was 12.2% for 18 to 24 year olds compared with 40.5% for people aged 65 and over. This compares with 19.3% not knowing among 11 to 18 year olds, as reported above. The proportion who inaccurately thought that vaping and smoking were equally harmful seemed to decline with age, going from 40.5% of 18 to 24 year olds to 23.1% of smokers aged 65 and over. In relation to gender, 38.6% of females who smoked inaccurately thought that vaping was equally harmful to smoking compared with 26.5% of male smokers, while 37.9% males accurately thought that vaping was less harmful than smoking compared with 29.7% females. Smokers from less advantaged groups (social grades C2DE) had greater misperceptions of vaping relative harms compared with those from more advantaged groups (social grades ABC1) (table 4).

Table 4.: Harm perceptions about vaping among current smokers by age, gender, region, social grade and ethnicity, England 2021 (STS, weighted percentage, unweighted counts)

	More harmful than regular cigarettes % (n)	Equally harmful % (n)	Less harmful than regular cigarettes % (n)	Don't know % (n)
Total	11.9 (369)	32.0 (1042)	34.1 (1061)	22.0 (685)
Age				
18 to 24	12.7 (43)	40.5 (142)	34.6 (112)	12.2 (40)
25 to 34	10.6 (55)	34.4 (174)	35.7 (180)	19.3 (88)
35 to 44	14.1 (48)	33.4 (122)	34.6 (135)	17.9 (66)
45 to 54	13.2 (48)	29.3 (108)	35.5 (133)	21.9 (84)
55 to 64	11.3 (34)	27.3 (83)	35.7 (113)	25.8 (77)
65+	9.4 (35)	23.1 (83)	27.0 (98)	40.5 (145)
Gender				
Male	11.4 (130)	26.5 (318)	37.9 (448)	24.2 (290)
Female	12.4 (133)	38.6 (394)	29.7 (323)	19.3 (210)
Region				
North	12.0 (73)	33.2 (205)	33.3 (213)	21.4 (136)
Midlands	12.4 (52)	30.8 (130)	29.9 (129)	26.9 (111)
South	11.6 (138)	31.8 (377)	36.0 (429)	20.6 (253)
Social grade				
ABC1	9.2 (101)	30.7 (337)	41.5 (429)	18.6 (205)
C2DE	13.6 (144)	33.2 (330)	29.4 (300)	23.8 (252)
Ethnicity				
White	11.6 (227)	31.5 (617)	34.7 (694)	22.2 (445)
Black and minority ethnic groups	14.1 (36)	35.3 (90)	30.9 (72)	19.7 (48)

Notes: Age 18+. Unweighted base for age, gender, region = 2,246; Social grade = 2,098; Ethnicity = 2,229. Current smokers included people who said that they smoked daily or that they smoked, but less than daily. STS data available from January to September 2021.

The ASH-A survey asked a question of all adult current smokers and vapers about what portion of the health risks of smoking come from nicotine in cigarettes.

Overall, few (13.9%) current smokers and vapers had accurate perceptions believing that none or a very small amount of the risk of smoking were due to nicotine, with 23.9% inaccurately reporting under half the risk, 17.3% inaccurately reporting around half the risk, 26.9% much more than half or nearly all the risk, and 18.1% reporting that they did not know (table 5).

A higher proportion of male participants (17.7%) accurately perceived that none or a very small amount of the risk from smoking comes from nicotine compared with female smokers and/or vapers (9.3%) (table 5). This accurate perception was also more prevalent among smokers and/or vapers from more advantaged (16.3%, ABC1) than less advantaged (11.9%; C2DE) social grades, and among those from white ethnic backgrounds compared with people from black and minority ethnic groups (14.9% and 9.5%, respectively) (table 5). There was a notable gradual increase in accurate nicotine harm perceptions depending on participants' experience with vaping—10.8% of current smokers, 15.6% of smokers and vapers, and 20.3% of current vapers reported that none or a very small amount of the health risks from smoking come from nicotine in tobacco cigarettes (table 5).

Table 5. Proportion of health risk of smoking perceived to be caused by nicotine, among current smokers and current vapers by age, gender, region, social grade, ethnicity, smoking status and vaping status, England 2021 (ASH-A, weighted percentage, unweighted counts)

	None or very small risk % (n)	Under half the risk % (n)	Around half the risk % (n)	Much more than half or nearly all the risk % (n)	Don't know % (n)
Total	13.9 (243)	23.9 (419)	17.3 (283)	26.9 (451)	18.1 (309)
Age					
18 to 24	9.1 (15)	28.2 (45)	14.9 (23)	26.5 (40)	21.2 (33)
25 to 34	14 (38)	24 (73)	20.0 (55)	25.2 (65)	16.8 (49)
35 to 44	12.9 (49)	24.5 (95)	17.1 (60)	24.7 (94)	20.8 (78)
45 to 54	16.7 (53)	24.4 (76)	14.0 (41)	27.7 (81)	17.2 (53)
55+	14.4 (88)	21.8 (130)	18.2 (104)	29.5 (171)	16.2 (96)
Gender					
Male	17.7 (164)	23 (210)	16.4 (133)	24.6 (204)	18.3 (157)
Female	9.3 (79)	24.9 (209)	18.3 (150)	29.7 (247)	17.8 (152)
Region					
North	14.2 (71)		15.2 (73)	27.0 (131)	20.7 (102)
Midlands	14.6 (49)	23 (116)	16.2 (51)	32.7 (101)	15.5 (54)
South	13.5 (123)	21 (69)	18.7 (159)	24.9 (219)	17.6 (153)
Social grade					
ABC1	16.3 (140)	27.4 (241)	16.6 (135)	23.3 (195)	16.4 (144)
C2DE	11.9 (103)	21 (178)	17.8 (148)	29.9 (256)	19.5 (165)
Ethnicity					
White	14.9 (218)	25.1 (366)	16.1 (226)	26.2 (364)	17.7 (252)
Black and minority ethnic groups	9.5 (19)	19.9 (45)	22.9 (47)	30.9 (68)	16.8 (37)

	None or very small risk % (n)	Under half the risk % (n)	Around half the risk % (n)	Much more than half or nearly all the risk % (n)	Don't know % (n)
Smoking and vaping status					
Current vaping	20.3 (99)	28.3 (142)	15.8 (73)	20.6 (93)	15.0 (70)
Current smoking	10.8 (109)	22.7 (229)	18.3 (174)	30.7 (300)	17.5 (177)
Current dual use	15.6 (35)	21.9 (47)	18.1 (36)	25.6 (55)	18.8 (40)

Notes: Age 18+. Unweighted base for age, gender, region and social grade = 1705; ethnicity = 1642; smoking and vaping status = 1679. Current vaping included people who currently vape every day and those who currently vape, but not every day. Dual users included people who currently vape daily or non-daily and smoke cigarettes daily or non-daily. Current smokers included daily and non-daily cigarette smokers.

15.5 Systematic review of vaping harm perceptions: examining interventions to change them, and longitudinal associations with vaping and smoking behaviours

Review questions

This systematic review addressed 2 research questions:

1. What interventions have been effective in changing vaping harm perceptions?

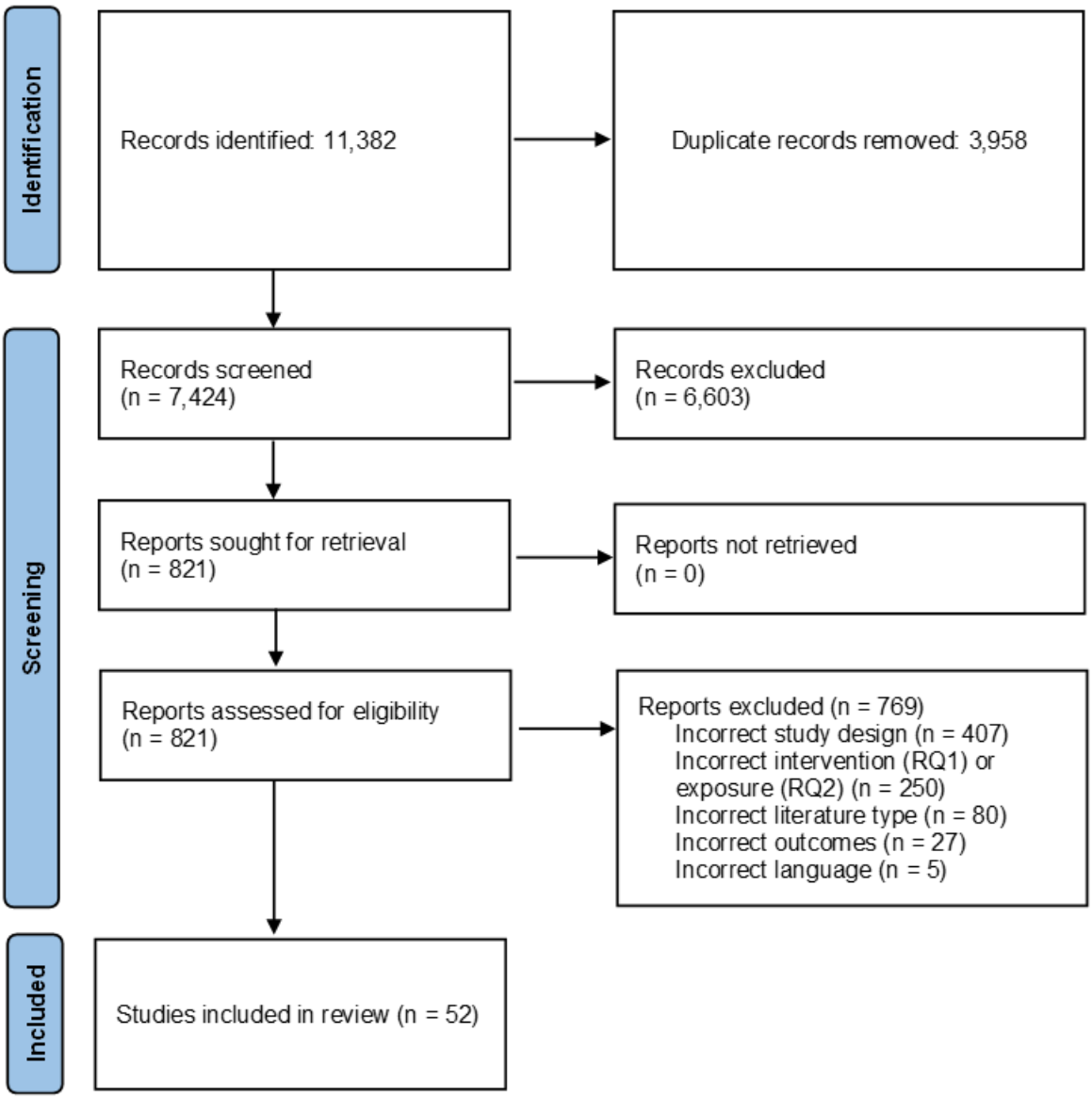
2. To what extent are vaping harm perceptions predictive of any changes in vaping and smoking behaviours?

Results

Study selection

The database searches identified 7,424 records after duplicates were removed. Independent screening by 2 reviewers identified 821 articles for full text screening, of which 52 articles were eligible for inclusion in the review (Figure 10). Of these 52 articles, 32 addressed RQ1 (2-33) and 21 assessed RQ2 (33-53). One article addressed both RQ1 and RQ2 (33).

Figure 10. PRISMA flow chart



Research question 1: what interventions have been effective in changing vaping harm perceptions?

Study characteristics

We identified 32 published articles that met our inclusion criteria for RQ1 (2-33). Study characteristics are shown in table 6.

Of these 32 articles, 29 were from unique studies, and 3 were from the same study (11, 14, 15).

Of these 32 articles, 27 were from the US (3-19, 21, 23, 26-33), 4 were from the UK (2, 22, 24, 25), and one from the UK and US (20).

Nineteen studies took place via online settings (5, 6, 8-15, 20, 21, 23-25, 27, 28, 31, 32), 4 in University or Community College settings (4, 7, 22, 26), one in university and online settings (19), 4 in school or after school settings (16, 17, 29, 33), one in a clinical laboratory (3), one in a US Air Force Base (18), and one in household settings (2). One study did not report the setting (30).

Sample sizes ranged from 36 (3) to 3,215 (2). Seventeen studies were among adults (age 18+, or 16+ for 2 studies (2, 24), or where a range was not given, the mean age >36 years) (2, 3, 6, 10-16, 19, 20, 24, 25, 27, 31, 32), 8 among young adults (age range 18 to 30 years, or where a range was not given, the mean age <25 years) (4, 5, 7, 8, 18, 22, 23, 26), and 7 among youth (age 10-19, or where a range was not given, mean age <17 years, or school students) (9, 17, 21, 28-30, 33). The proportion of males ranged from 18% (8) to 85% (30).

Fourteen studies used a randomised design (4, 6-8, 10, 11, 13-15, 20, 21, 25-27), while 12 studies used a non-randomised design (one multi-group and non-randomised (12), 11 one-group (3, 5, 9, 16-19, 22, 28-30)), 4 used a repeated cross-sectional design (2, 24, 31, 32), and 2 used a cohort design (23, 33).

Twenty-five studies used a pre-post design with no longitudinal follow-up (33-37, 39, 41-45, 47-51). For the 2 cohort studies, follow-up was 3 (23) or 6 months (33).

Risk of bias

Quality of the randomised and non-randomised experimental studies was generally low (Appendix 2).

Among the 14 randomised studies, 13 had ‘some concerns’ (4, 6-8, 10, 11, 13-15, 20, 21, 26, 27) and only one was considered ‘low risk’ (25). Most concerns were attributable to high prevalence of missing data and a lack of evidence that missing data may have biased the outcome, as well as a lack of a pre-specified analysis plan.

Among the 12 non-randomised studies, all were considered to have ‘serious risk of bias’, but not critical risk of bias in any domain (3, 5, 9, 12, 16-19, 22, 28-30). All studies had serious risk of bias on the confounding domain, due to lack of adjustment for key confounders (for example, age, gender). Only one study adjusted for confounders (perceived absolute smoking risk, response efficacy of vaping to help quit smoking, and response efficacy of vaping to reduce smoking) but did not adjust for demographics such as age or gender (12).

Quality of the cross-sectional studies was generally acceptable. Among the 4 repeated cross-sectional studies, scores on the adapted 8-star Newcastle Ottawa Scale ranged from 5 (2, 24, 31) to 6 (32), with higher scores indicating lower risk of bias.

Quality of the 2 cohort studies was low (23, 33), with scores on the adapted 5-star Newcastle Ottawa Scale being 2 (23) and 3 (33) both indicating high risk of bias (≤3 stars indicates high risk of bias).

Description of interventions and outcomes

Study interventions and outcomes are shown in table 7 to table 12.

Thirteen articles (from 10 studies) described interventions involving written information about vaping (3-15); 3 articles described data from the same study (11, 14, 15). Four articles described interventions involving education about vaping, such as educational workshops (16-18) or educational videos (19). Five articles described interventions involving exposure to mass media campaigns (21, 23, 24) or advertisements (20, 22). Three articles described interventions involving packaging/warning labels (25-27). Three articles described interventions involving video games (28-30). This review also included articles that evaluated the impact of EVALI on changing vaping harm perceptions, of which 4 articles were identified (2, 31-33).

The interventions focused on a range of harms associated with vaping, and some interventions focused on several types of vaping harms. Of the 32 articles, 13 focused on relative harms of vaping compared with smoking (10 lower relative harms (3, 6, 11-13, 15, 20, 22, 24, 25); 2 equal harm (21, 23); one both lower and equal harms (27)); 14 focused on absolute harms of vaping (5, 7, 9, 10, 16-19, 21, 23, 26, 27, 29, 30) (4 of which focused on risk of developing specific diseases/health outcomes (16, 21, 23, 27)), 9 focused on addictiveness of vaping and/or nicotine (4, 5, 7, 9, 11, 17, 21, 25, 26), 3 focused on providing accurate information about nicotine (11, 14, 24), one focused on harms of secondhand vapour (4), and one focused on both benefits and harms of vaping (8). Two focused on correcting vaping ‘misperceptions’ but did not provide detail on what were considered misperceptions (19, 28).

Outcomes also consisted of several types of vaping harms, and some studies assessed multiple outcomes. Eighteen studies assessed absolute risk of harm from vaping (that is, general harms of vaping not in relation to smoking) (3-5, 8, 9, 12, 17, 18, 20-22, 25, 27-32); 7 assessed the perception that vaping would cause specific diseases or health ailments (6, 9, 16, 21, 23, 26, 27); and 11 studies assessed concern about dependency or addiction to vaping (3, 4, 9, 11, 14, 17, 25, 26, 28, 30, 33). These sets of outcomes are grouped together as ‘absolute vaping harms’ in the relevant tables. Sixteen studies assessed relative risk of harm from vaping compared to smoking (7-15, 19, 23, 24, 26, 31, 33, 54), identified as ‘relative vaping risks’ in relevant tables. Three assessed the perception of nicotine harms (11, 14, 23), identified as ‘nicotine risk’ in relevant tables.

Findings

We have grouped the findings into the following sections: interventions involving written information about vaping, interventions involving education about vaping, interventions

involving mass media campaigns or advertisements, interventions involving packaging/warning labels, interventions involving video games, EVALI.

Interventions involving written information about vaping

Table 7 shows articles that assessed the associations between interventions involving written information about vaping and changes in vaping harm perceptions.

Six articles (from 5 studies), all among adults, provided written information about the reduced risk of vaping relative to smoking (3, 6, 11-13, 15). Of these 6 articles, 5 found statistically significant associations with harm perceptions of vaping, although this was not significant for all the conditions/groups tested (6, 11-13, 15). Specifically, exposure to written information about the reduced risk of vaping relative to smoking decreased the perception that vaping would cause specific health ailments (for example, lung cancer, emphysema, stroke) (6) and increased accurate relative harm perceptions (perceiving that vaping is less harmful than smoking (11, 13); that switching from smoking to vaping would reduce health harms (15)), although exposure was not statistically significantly associated with changes in perceptions of the addictiveness of nicotine in one of these studies (11). Another study found that exposure to written information about the reduced risk of vaping relative to smoking increased perceptions that vaping is less harmful than smoking (relative harm) but – unexpectedly – also increased perceptions that vaping is harmful (absolute harm) among some, but not all, smoker subgroups (12). Only one study, which had small sample size (n=36), found no statistically significant association between exposure to written information about the reduced risk of vaping relative to smoking and changes in vaping harm perceptions (3).

Three articles (2 from the same study) provided written information about nicotine – 2 of which aimed to disseminate accurate information about nicotine via a fact sheet among adults (11, 14), and one of which aimed to inform young adults about the risks of nicotine (4). Of these, all 3 found statistically significant associations with changes in vaping or nicotine harm perceptions (4, 11, 14). Two articles, both from the same study, describe exposing adults to a nicotine fact sheet stating that nicotine was not the main cause of harm from smoking, but that it was a poison at very high doses, was not safe to use in pregnancy, could harm the adolescent brain and was addictive (11, 14). The study tested the fact sheet, either alone (14) or in combination with a message about vaping risks relative to smoking (for example, ‘switching to e-cigarettes completely can reduce your risk for health issues’) (11) and found that the nicotine fact sheet specifically decreased the perception that nicotine causes most of the smoking-related health problems, but – perhaps not unexpectedly – was not statistically significantly associated with the perception that vaping is less harmful than smoking (relative harm) or the perception that nicotine is the main addictive substance in tobacco. The third study exposed young adults to written information about nicotine addiction, secondhand vapour, or dermal absorption of nicotine, and found that exposure increased perception of absolute vaping harms (for

example, that people risk harming themselves if they vape every day) and nicotine addiction (4).

Five studies provided written information about absolute harms of vaping, sometimes including addictiveness/nicotine risks – 3 of which provided written information about absolute harms of vaping and addictiveness designed to deter use among young adults (5, 7) or youth (9), one of which provided written information about the benefits, harms, or both benefits and harms to young adults (8), and one of which highlighted the uncertainty of vaping risks, compared with absolute harms of vaping to adults (10). Of these 5 studies, 3 found statistically significant associations with changes in vaping or nicotine harm perceptions: 2 among youth or young adults designed to deter use (5, 9) and the one study among adults which highlighted the uncertainty of vaping risks (10). The only study among adults, found that exposure to an uncertainty message (for example 'Not enough scientific evidence exists to say for sure how using electronic vaping products could affect your health in the short or long term'), compared with a message about the harms of vaping (for example 'Studies have shown that the liquids in vaping products contain chemicals that are harmful when inhaled'), decreased the perception that vaping is harmful to health (absolute harm) (10). The second study found that exposure to written information about vaping harms (harmful chemicals, nicotine may harm teen brain development) and addictiveness (nicotine is an addictive chemical) increased perceptions that vaping is equally or more harmful than smoking (relative harm), as well as absolute harm perceptions including risks of specific diseases (9). The third study found that exposure to written information about JUUL (nicotine content, ingredients, marketing to youth, and information that JUUL use may benefit smokers but harm non-smokers) increased perceptions of the absolute risk of JUUL use to the self and bystanders. The remaining 2 studies assessed the impact of written information about vaping harms and addictiveness designed to deter use among young adults (for example, highlighting that e-cigarettes contain harmful chemicals and that nicotine is an addictive chemical) or written information about the benefits and harms of vaping to young adults but found no statistically significant association of the intervention with relative or absolute vaping harm perceptions overall (7, 8).

Interventions involving education about vaping

Table 8 shows articles that assessed the associations between interventions involving education about vaping and changes in vaping harm perceptions.

Three studies involved educational workshop interventions designed to deter e-cigarette use among adults (16), young adults (18), and youth (17) through providing information about the absolute risks of vaping (for example, heart disease, cancers, respiratory diseases) and addictiveness. Of these 3 studies, 2 found statistically significant associations with increased vaping harm perceptions (17, 18). Specifically, one found that a brief tobacco/vaping intervention workshop fostering negative attitudes towards nicotine

products and increasing ‘knowledge regarding the health consequences of nicotine use’ increased young adults’ perceptions that vaping is harmful to health (18). Another found that a brief educational presentation about vaping, associated harms and nicotine addiction, increased youths’ perceived harms from vaping and vaping addictiveness perceptions (17). The third study, which was among adults and had small sample size (n=41), found no statistically significant association between taking part in an educational workshop providing information about specific diseases resulting from vaping and perceptions of developing specific diseases from vaping (16).

One study exposed university students to an educational video providing information about the ‘health effects of vaping’ and addressing ‘vaping misperceptions’, and found that exposure increased the perceived relative harm of vaping to smoking (19). However, the study did not provide a definition of the health effects of vaping or vaping misperceptions.

Interventions involving mass media campaigns or advertisements

Table 9 shows articles that assessed the associations between interventions involving mass media campaigns or advertisements and changes in vaping harm perceptions.

Three studies described interventions involving exposure to mass media campaigns (21, 23, 24). Of these, 2 found statistically significant associations with vaping harm perceptions (21, 23). Specifically, the first study, evaluating the impact of a youth vaping prevention campaign, found that the campaign increased youths’ perceptions of absolute vaping harms and the risk of developing vaping-related diseases (21). The second study found that young adults’ self-reported exposure to refuting incorrect information about vaping (for example, exposure to information refuting the claim that ‘e-cigarettes are just as dangerous as actual cigarettes for health’), but not exposure to incorrect information about vaping, was statistically significantly associated with a reduction in perceptions of relative harms, absolute harms, and risk of developing vaping-related diseases (23). The third study, evaluating the impact of a regional campaign which highlighted that vaping is less harmful than smoking among adults, found no statistically significant association with harm perceptions of vaping relative to smoking (24).

Two additional studies assessed different vaping product advertisements that promoted vaping (some in comparison with smoking) among adults or young adults (20, 22). Of these 2, one found a statistically significant association with vaping harm perceptions (20); specifically, perceptions of vaping as ‘healthy’ (absolute harm) increased after exposure to advertisements promoting vaping (for example, e-cigarettes as a smoking cessation tool, healthier than cigarettes, aesthetically pleasing, celebrity endorsed, sporty) among adults (20). The other study, among young adults, found no statistically significant association between exposure to advertisements comparing vaping to smoking (for example, ‘a healthier option to smoking’; ‘no tobacco, no smoke, just pure satisfaction for smokers’) and changes in the perception of vaping as harmful to health (absolute harm) (22).

Interventions involving packaging/warning labels

Table 10 shows articles that assessed the associations between interventions involving packaging/warning labels and changes in vaping harm perceptions.

Two studies involved exposure to warning labels focusing on the relative risk of vaping compared with smoking (25, 27). Of these 2, only one found statistically significant associations between a reduced risk warning label and absolute vaping harm perceptions (including addictiveness) (25), while the other did not but instead found a statistically significant association between an 'equal' risk warning label and absolute vaping harm perceptions (27). The first study, among adult non-vapers, found that exposure to a reduced risk warning label (for example, 'Use of this product is much less harmful than smoking') decreased perceptions of the harms of vaping (absolute harms) and perceptions of vaping as addictive (25). The second study, among adult non-smokers and non-vapers, found no statistically significant association between exposure to a reduced risk warning label ('Warning: No tobacco product is safe, but this product presents substantially lower risks to health than cigarettes') and perceptions of the harms of vaping (absolute harms) including risk of cancer (27); however, this same study did find that exposure to an 'equal' risk warning label ('Warning: This product is not a safe alternative to cigarettes') increased perceptions of the harms of vaping (absolute harms) including risk of cancer (27). Neither study tested whether the labels affected relative harm perceptions.

Two studies involved exposure to warning labels focusing on the absolute risk of vaping (26, 27). Of these 2, only one found statistically significant associations with absolute vaping harm perceptions (including addictiveness) (27) and the one study assessing relative harm perceptions (including addictiveness) found no statistically significant association (26). The first study, among adult non-smokers and non-vapers, found that exposure to a warning label with a picture of a mouth sore and stating 'Warning: This product can cause mouth cancer' increased perceptions of the harms of vaping (absolute harms) including risk of cancer (27). The second study, among young adults, found that exposure to a warning label with several statements about vaping, for example 'Inhalation of this product may aggravate existing respiratory conditions' found no statistically significant association with absolute harm perceptions of vaping, including risk of developing specific diseases and nicotine addiction, or relative harm perceptions of vaping compared with smoking including addictiveness (26).

Two studies involved exposure to warning labels focusing on the addictiveness of nicotine (25, 26). Of these 2, both found statistically significant associations with absolute vaping harm perceptions (including addictiveness) (25, 26) but the one study assessing relative harm perceptions (including addictiveness) found no statistically significant association (26). The first study, among adult non-vapers, found that exposure to nicotine addiction warning labels that were implemented in the EU and UK (for example, 'This product contains nicotine which is a highly addictive substance' increased perceptions of the

harms of vaping (absolute harm) and perceptions of vaping as addictive (25). The second study, among young adults, found that exposure to a nicotine addiction warning label stating ‘WARNING: This product contains nicotine derived from tobacco. Nicotine is an addictive chemical’ increased absolute harm perceptions of vaping, including risk of developing specific diseases and nicotine addiction, but did not change relative harm perceptions of vaping compared with smoking including relative addictiveness (26).

One study, among adult non-smokers and non-vapers, also assessed another label which stated ‘FDA Approved’ on vaping product packaging, but found no statistically significant association with perceptions of the harms of vaping (absolute harm) including risk of cancer (27).

Interventions involving video games

Table 11 shows articles that assessed the associations between interventions involving video games and changes in vaping harm perceptions.

Three studies described interventions involving video games aimed to prevent youth vaping and help youth to develop skills to refuse vaping (28-30). All 3 found statistically significant associations with vaping harm perceptions (28-30). Specifically, perceptions of vaping harms (absolute harms) increased after playing the video games in all 3 studies (28-30). The perceived addictiveness of vaping also increased in one (28) of 2 (28, 30) studies after playing the video games, where these outcomes were assessed.

EVALI

Table 12 shows articles that assessed the associations between EVALI and changes in vaping harm perceptions.

Four studies evaluated whether vaping harm perceptions changed after EVALI, 3 of which were among adults and one among youth (2, 31-33). All 4 found statistically significant associations with vaping harm perceptions (2, 31-33). Specifically, perceptions of the harms of vaping (absolute harms) (31, 32), the harms of vaping relative to smoking (31, 33, 54), and perceived addiction of vaping relative to smoking (33) all increased after EVALI.

Table 6. Study characteristics of articles that addressed research question 1: what interventions have been effective in changing vaping harm perceptions? Studies are organised by intervention type

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Interventions involving written information about vaping				
Bono et al. (2019) (3)	US, Clinical Laboratory Dates of data collection: 02/2015 - 07/2016	n=36 Adults (age 18-55 years, median age = 36 years [IQR= 27-49.5 years]) 75% male, 25% female Had to be current smokers who had not vaped weekly or more for at least a month to be eligible One-group experiment	Massey Cancer Center Pilot Project Program, the VCU Center for Clinical and Translational Research Endowment Fund, NIDA, NIH, FDA CTP COIs: None declared	ROBINS-I: Serious risk
Calabro et al. (2019) (4)	US, Community College 09/2016 - 10/2016	n=95 Young adults, community college students (age 18-24 years, mean=20.8 years [SD=1.8]) 54% male 16% smoked, and 11% vaped, in the past 30 days Randomised experiment	TCORs, NIH, NCI COIs: None declared	RoB2: Some concerns
Carpenter et al. (2021) (5)	US, Online 01/2019 - 04/2019	n=947 Young adults (age 18-30 years, mean=26.1 years [SD=3.0]) 58% male 100% tobacco/nicotine product users: 67% daily/almost daily smokers, 20% weekly smokers, 8% monthly smokers, 4% yearly smokers; 10% used JUUL in the past 30 days, 49% used other e-cigarettes One-group experiment	Oklahoma State University Graduate Research Fellowship, National Center for Advancing Translational Sciences, NIDA COIs: None declared	ROBINS-I: Serious risk

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
DeHart et al. (2019) (6)	US, Online 11/2017	n=157 Adults (mean=36.6 years [SD=11.5]) 66% male Had to smoke at least 10 cigarettes per day to be eligible (FTND score mean = 11.0 [SD=1.4]); vaping status NR, but both vapers and never vapers could participate Randomised experiment	Fralin Biomedical Research Institute at VTC, NIH, NIDA, FDA COIs: Author(s) are principal of HealthSim, LLC; Notifius, LLC; BEAM Diagnostics, Inc.; and a partner for Red 5 Group, LLC, and serves on the scientific advisory board for Sober Grid, Inc.; Ria Health; US WorldMeds, LLC; and is a consultant for Alkermes, Inc.	RoB2: Some concerns
Keating (2018) (7)	US, University 03/2017 - 04/2017 and 03/2017 - 04/2017	n=192 Young adults, university students (age 18-53 years, mean=24.3 years [SD=6.2])1 56% female1 52% reported using a cigarette, 26% reported using cigarettes at least once in the past week; 33% reported using an e-cigarette, 22% reported using an e-cigarette at least once in the past week1 Randomised experiment	No funding or COIs declared	RoB2: Some concerns
Majumdar et al. (2019) (8)	US, Online 2016 (month not stated)	n=191 Young adults, university students (age 18-25 years) 18% male, 81% female Smoking and vaping status NR Randomised experiment	No funding or COIs declared	RoB2: Some concerns

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Noar et al. (2019) (9)	US, Online 2014 - 2015 (month not stated)	n=61 Youth (age 14-18 years, mean=16.33 years [SD=0.9] 48% male, 48% female, 5% gender non-conforming 3% past 30-day smokers, 28% ever but not past 30-day smokers; 17% past 30-day vapers, 30% ever but not past 30-day vapers One-group experiment	NCI and FDA CTP COIs: None declared	ROBINS-I: Serious risk
Pepper et al. (2019) (10)	US, Online Dates of data collection NR	n=2508 Adults (age 18+) 47% male, 53% female 49% current smoker, 50% non-smoker; 42% vape every/some days, 58% vape rarely/not at all Randomised experiment	Funding: Research Triangle Institute (RTI) International. COIs: None declared	RoB2: Some concerns
Yang & Popova (2020) (11)	US, Online Dates of data collection NR	n=1528 Adults (age 18+ years) 46.3% male, 53.1% female, 0.5% transgender, 0.1% other Had to be current smokers or recent former smokers to be eligible (96.5% current, 3.5% former); 46% current vapers, 24% former vapers, 30% never vapers Randomised experiment	NIDA, NIH, NCI, FDA CTP COIs: None declared	RoB2: Some concerns
Yang et al. (2018) (12)	US, Online Dates of data collection NR	n=580 Adults (age 18-64 years) 59% to 77% female depending on the group (gender of overall sample not reported) Had to be current smokers; vaping status NR Non-randomised experiment	NIDA, NCI, NIH, FDA CTP COIs: None declared	ROBINS-I: Serious risk

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Yang et al. (2019) (13)	US, Online Dates of data collection NR	n=1400 Adults (age 18+ years) 35% female, 47% male, remainder unspecified Had to be current smokers or recent former smokers to be eligible (61% daily, 9% former); 34% current vapers, 23% ever but not current vapers, 44% never vapers Randomised experiment	NIDA, NIH, FDA CTP, NCI COIs: None declared	RoB2: Some concerns
Yang et al. (2020) (14)	US, Online Summer 2018	n=756 Adults (age 18+ years) 45% male, 54% female, 0.9% transgender, 0.1% other Had to be current smokers or recent former smokers to be eligible Randomised experiment	NIDA, NIH, FDA CTP, NCI COIs: None declared	RoB2: Some concerns
Yang et al. (2021) (15)	US, Online Dates of data collection NR	n=761 Adults (aged 18+ years) 48% male, 52% female, 0.5% transgender Had to be current smokers or recent former smokers to be eligible (96% current, 4% former); 46% current vapers, 24% former vapers, 29% never vapers Randomised experiment	NIDA, NIH, FDA CTP COIs: None declared	RoB2: Some concerns
Education about vaping				

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Baer et al. (2021) (16)	US, School 08/2019	n=41 Adults, middle and high school staff (20-65 years, mean=42.3 years [SD=11.4]) 49% male, 49% female, 2% NR 5% current smokers, 39% experimented but not a current smoker, 44% never smokers, 12% former smokers; 88% never vapers, 12% experimented but not a current vaper One-group experiment	No funding or COIs declared	ROBINS-I: Serious risk
Gaiha et al. (2021) (17)	US, School 02/2019 - 05/2019	n=2889 Youth, middle and high school students (age NR) Gender NR 1% had tried smoking only, 11-18% (depending on age) had tried vaping only, 8-14% % (depending on age) had tried both smoking and vaping One-group experiment	California's Tobacco-related Disease Research Program and the Alabama Department of Public Health Youth Tobacco Prevention Program Grant. COIs: Author(s) founded and direct the resource used for the intervention in this study.	ROBINS-I: Serious risk
Little et al. (2016) (18)	US, Air Force base 10/2014 - 03/2015	n=1055 Young adults, military personnel (mean=20.1 years [SD=2.5]) 77.4% male 12% smoked, 9% vaped One-group experiment	No funding declared COIs: Author(s) have received funding from JHP Pharmaceuticals, Orexigen, and Pfizer	ROBINS-I: Serious risk
Sergakis et al. (2019) (19)	US, University and online Dates of data collection NR	n=115 Adults, university students (age 19-36 years, mean=21 years) 24% male, 82% female Smoking and vaping status NR One-group experiment	No funding or COIs declared	ROBINS-I: Serious risk
Mass media campaigns or advertisements				

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Booth et al. (2019) (20)	UK and US, Online 12/2015 - 02/2016	n=765 Adults (age 18-65 years, mean=36 years [SD=11.6]) 47% male, 52% female, 0.5% other 33% smoked occasionally, often, or always, 67% never smoked; 32% vaped occasionally, often, or always, 68% never vaped Randomised experiment	CRUK COIs: Author(s) have consulted on life insurance (Pacific Life) and have received funding from Allen Carr's Easyway	RoB2: Some concerns
England et al. (2021) (21)	US, Online Dates of data collection NR	n=268 Youth (age 11-19 years, mean=14.8 years) 44% male, 54% female Smoking and vaping status NR Randomised experiment	Virginia Foundation for Healthy Youth (VFHY) COIs: None declared	RoB2: Some concerns
Ratneswaran et al. (2019) (22)	UK, University 03/2015	n=106 Young adults, university students (eligible age 18-80, mean age 22 ± 2 years) 66% male, 34% female 32% current smokers, 54% non-smokers, 14% former smokers; 16% current vapers, 77% non-vapers, 7% former vapers One-group experiment	No funding or COIs declared	ROBINS-I: Serious risk
Tan et al. (2015) (23)	US, Online 01/2014 with follow-up 3 months later	n=411 Young adults, university students (mean=20.3 years [SD=1.5]) 31% male, 67% female 87% nonsmoker, 3% former smoker, 8% current smoker; mean frequency of vaping in the past 30 days was 0.4 days [SD=2.0] Cohort study	No funding or COIs declared	NOS score 2/5 (high risk)

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Tattan-Birch et al. (2020) (24)	UK, Online 12/2017 - 01/2018 and 02/2018 - 03/2018	n=1637 Adults (age 16+) Gender NR 37% smokers; vaping status NR Repeated cross sectional	CRUK, ESRC COIs: Author(s) have received funding from Pfizer and acted as paid reviewer for grant awarding bodies and as a paid consultant for health care companies.	NOS score 5/8

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Packaging/warning labels				
Kimber et al. (2020) (25)	UK, Online Dates of data collection:12/2018 - 01/2019	n=2495 Adults (age 18+ years) 47% male, 53% female 44% daily smokers, 5% occasional smokers, 49% non-smokers; 100% non-current vapers (71% of which had never vaped) Randomised experiment	Funding: CRUK COIs: Author(s) have received funding from Allen Carr's Easyway Ltd, provided consultancy services to UK life insurers and for the pharmaceutical industry, acted as an expert witness on cases relating to vaping, and conducted research for independent electronic cigarette companies.	RoB2: Low risk
Lee et al. (2018) (26)	US, University 09/2015 - 10/2015	n=666 Young adults, university students (age 18-25 years, mean=19.9 years [SD=1.55]) 44% male, 56% female Smoking status NR; 70% had ever tried vaping Randomised experiment	No specific grant from funding agencies in the public, commercial, or not-for-profit sectors. COIs: None declared	RoB2: Some concerns
Popova & Ling (2014) (27)	US, Online Dates of data collection NR	n=483 Adults (age 18+ years, mean=47 years) 44% male, 56% female Had to not be established smokers or vapers to be eligible Randomised experiment	NCI COIs: None declared	RoB2: Some concerns

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Video games				
Hieftje et al. (2021) (28)	US, Online 10/2017 and 04/2018	n=560 Youth, school students (age 10-13 years, mean=11.9 years [SD=2.0]) 54% female Smoking and vaping status NR One-group experiment	CVS Health Foundation, National Heart, Lung, and Blood Institute, CTSA, National Center for Advancing Translational Science (NCATS) COIs: None declared	ROBINS-I: Serious risk
Pentz et al. (2019) (29)	US, After school Dates of data collection NR	n=80 Youth, students in a community afterschool programme (age 11-14 years) 39% male, 61% female 1% lifetime smoking, 4% lifetime vaping One-group experiment	NIH, FDA. Vanderbilt Center for Tobacco, Addiction, and Lifestyle, and VCREATE, the Vanderbilt Clinical Cardiovascular Outcomes Research and Trial Evaluation. COIs: None declared	ROBINS-I: Serious risk
Weser et al. (2021) (30)	US, setting NR 08/2018 - 09/2018	n=47 Youth, school students in an afterschool programme (mean=14.2 years [SD=0.91]) 85% male, 9% female Smoking status NR; 9% vapers One-group experiment	Oculus COIs: Author(s) have ‘a significant relationship’ with the company who developed the intervention.	ROBINS-I: Serious risk
EVALI				
Alber et al. (2021) (31)	US, Online 07/2019 and 10/2019	n=1057 Adults (age 18+ years, mean=39.8 years [SD=14.8]) 49% male, 51% female, 0.6% other 58% had smoked at least 100 cigarettes in life; 46% had never vaped Repeated cross sectional	William and Linda Frost Fund in the Cal Poly College of Science and Mathematics COIs: None declared	NOS score 5/8

Authors and year	Country, setting, and data collection period	Participants and study design	Funding, conflicts of interest (COI)	Risk of bias
Morgan et al. (2021) (32)	US, Online 08/2019 and 09/2019	n=1209 Adults (mean=46-48 years [SD=16.4-17.6]) 47-52% male, 48-53% female Had to be a current or former smoker to be eligible (54-58% current, 42-46% former); 33-34% current vaper, 19-20% former vaper, 47-48% never vaper Repeated cross sectional	NCI and FDA CTP COIs: None declared	NOS score 6/8
Moustafa et al. (2021) (33)	US, High schools Spring 2019 and Fall 2019	n=1539 Youth, school students (mean=16.7 years [SD=0.6]) 50% male, 50% female 3% smoked in the past 6 months, 97% had not; 11% vaped in the past 30 days, 89% had not Cohort study	NCI COIs: None declared	NOS score 3/5 (high risk)
Tattan-Birch et al. (2020) (2)	UK, Household 03/2016 and surveyed every 3 months until 12/2019	n=3215 Adults (age 16+, mean=43-44 years [SD=17-18]) 54-56% male, 44-46% female Had to be a current smoker to be eligible; vaping status NR Repeated cross sectional	CRUK, PHE COIs: Author(s) have received funding from Pfizer and Johnson & Johnson.	NOS score 5/8

Notes: [1] Demographics are provided among 404 participants at baseline (not only restricted to those 192 who completed follow-up and were included in analyses).

[2] Risk of bias was assessed for all studies using the Newcastle Ottawa Scale (NOS), with scores of >4 (out of 8) stars indicating low risk of bias and scores of ≤3 stars indicating high risk of bias.

NR = not reported.

CRUK = Cancer Research UK, CTP = Center for Tobacco Products, ESRC = Economic and Social Research Council, FDA = Food and Drug Administration, MRC = Medical Research Council, NCI = National Cancer Institute, NIDA = National Institute on Drug Abuse, NIH = National Institutes of Health, PHE = Public Health England, TCORS = Tobacco Centers of Regulatory Science.

Table 7. Associations between interventions involving written information about vaping and changes in vaping harm perceptions (research question 1)

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Bono et al. (2019) (3) US	**Written information about reduced harm of vaping relative to smoking.** 2 studies. In each, participants used a flavoured e-cigarette and were exposed to risk messages, resulting in 4 conditions per study (flavour*risk message). Participants completed all conditions within their study, each 48 hours apart. Study 1: Tobacco- and menthol-flavored e-cigarettes with a message stating e-cigarettes had “reduced harm relative to cigarettes”. Study 2: Unflavored and cherry-flavored e-cigarettes with a message stating e-cigarettes had “reduced exposure to carcinogens relative to cigarettes”. Messages were read aloud, presented on written cards, and displayed on the wall throughout sessions. **Adult current smokers who did not currently vape** (overall n=36: n=17 in study 1 and n=19 in study 2) **One-group experiment** with change assessed within person Adjusted for menthol vs. non-menthol own-brand cigarette preference.	**×** Concern about dependency/addiction and perceived harm of vaping to health did not differ by e-cigarette risk message or flavour (all p>.05).	N/A	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Calabro et al. (2019) (4) US	**Written information about nicotine addiction and secondhand vapour.** Participants were randomised to receive text messages about vaping across 3 topics: 1) nicotine addiction 2) secondhand vapour 3) dermal absorption of nicotine. Examples: “Ppl who don’t vape avoid nicotine addiction” “Ppl who vape e-cigs are haunted by nasty nicotine cravings that can take over their life! They are chained to an addiction demon!” **Young adults** (n=95) **Randomised experiment** with change assessed within person.	✓ The following perceptions increased after exposure: 1) People risk harming themselves if they use e-cigarettes every day (p=.002), 2) E-cigarettes are not a proven and safe way to quit (60.2% to 84.2%, p<.001), 3) E-cigarettes are not regulated by the government (FDA) (55.9% to 67.4%, p=.03), 4) Using e-cigarettes can lead to nicotine addiction (80.6% to 91.6%, p=.04), 5) Using e-cigarettes may lead people to try other products, including regular cigarettes (75.3% to 90.5%, p=.007), 6) When you “smoke an e-cigarette” you don’t	N/A	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
		know how much nicotine you are getting (54.8% to 73.7%, p=.01), 7) if e-cigarette liquid comes in contact with your skin it can be absorbed and cause health problems (57.0% to 83.2%, p<.001), 8) There can be risks to other nonusers if exposed to nicotine vapor exhaled by persons using e-cigarettes (66.3% to 84.2%, p=.004).		
Carpenter et al. (2021) (5) US	**Written information about JUUL (nicotine, absolute harms).** Participants were provided with a description of the JUUL device and characteristics including: (1) nicotine content (e.g., 'one JUUL pod is equivalent to approximately one pack or 200 puffs of a cigarette') (2) ingredients (nicotine, propylene glycol, glycerin, benzoic acid, flavorants) (3) JUUL's marketing strategies, including initial marketing to youth and the resulting FDA scrutiny	✓ Perceived absolute risk of JUUL use to the self (mean=5.1 [SD=3.0] to 5.7 [SD=3.1], t=-8.9; p<.001; d=0.20) and bystanders (4.0 [SD=3.2] to 4.8 [SD=3.3]; t=-11.4; p<.001; d=0.25) increased after	N/A	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
	(4) JUUL's rapid increase in e-cigarette market share (5) information regarding to whom JUUL use may be beneficial (smokers) vs. harmful (non-smokers). **Young adult tobacco/nicotine users** (n=947) **One-group experiment** with change assessed within person	exposure to information about JUUL.		
DeHart et al. (2019) (6) US	**Written information about reduced harm of vaping relative to smoking.** Participants were randomised to one of 4 conditions: 1) e-cigarette with authority bias (describes a friend of the reader who permanently switches to e-cigarettes after a COPD diagnosis and after physician recommends switching, and makes a recovery) 2) e-cigarette with social proof (describes a friend of the reader who permanently switches to e-cigarettes after a COPD diagnosis and after a second friend encourages the principal friend to switch by addressing the stigma of using ENDS, and makes a recovery 3) control condition 1 – CDC narrative (describes a woman who quits smoking after a COPD diagnosis) 4) control condition 2 – e-cigarette without biases (describes a friend of the reader	✓ Perception that vaping would cause specific health ailments including lung cancer, emphysema, and stroke (from 1 (very low risk) to 10 (very high risk)) decreased after reading the 2 intervention narratives: E-cigarette with authority bias (MD=-7.63, p< .001, d=0.35) E-cigarette social proof (MD=-7.18, p<.001, d=0.33). Findings for the	N/A	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
	who permanently switches to e-cigarettes after a COPD diagnosis and makes a recovery). For all but the CDC condition, the 'friend' described was matched to reader demographics on smoking behaviour, gender and age. **Adult smokers** (n=157) **Randomised experiment** with change assessed within person	control conditions were not reported.		
Keating (2018) (7) US	**Written information about vaping harms and addictiveness.** Participants were randomised to 1 of 3 conditions: 1) Norm ("Did you know that only about 10% of [university] students say they have ever used e-cigarettes? There aren't as many tobacco users on campus as you might think…") 2) Harm ("You probably know that cigarettes contain harmful chemicals, but did you know that e-cigarettes do as well? Among other things, e-cigarettes contain nicotine, which is an addictive chemical…") 3) Efficacy ("Avoiding cigarette use is not easy, but it is not impossible! [University] has a ton of effective resources that can help students quit smoking, saving you and your classmates from harmful smoke…" **Young adults** (n=192) **Randomised experiment** with change	× Perception of vaping harms showed little increase after exposure to text statements when aggregated across conditions (all five-point scale from strongly disagree to strongly agree): E-cigs contain harmful chemicals (M=4.4 [SD=0.8] to M=4.5 [SD=0.7], t=1.8, p=.079) It is easy to become addicted to e-cigs (M=4.1, [SD=0.9] to M=4.3 [SD=0.9],	× Perception that "smoking e-cigs" is better for you than smoking cigarettes (five-point scale from strongly disagree to strongly agree) showed little increase after exposure to text statements when aggregated across conditions (M=3.0 [SD=1.3] to M=3.0 [SD=1.2], t=0.06, p=.952) and an ANOVA indicated no significant main effects of time	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
	assessed within and between person	t=1.19, p=.237) "Smoking e-cigs" is bad for your health (M=4.5 [SD=0.8] to M=4.5 [SD=0.8], t=0.18, p=.857)	(F(1,188)=0.01, p=.930), condition (F(2,189)=0.88, p=.418), or time*condition (F(2,188)=0.12, p=.889)	
Majumdar et al. (2019) (8) US	**Written information about benefits and harms of vaping.** Participants were randomised 1 of 3 conditions: 1) positive (benefits of vaping) 2) negative (harms of vaping) 3) ambivalent (both benefits and harms). Analyses were split by whether participants were 'univalent' or 'ambivalent' in their thinking. **Randomised experiment** with change assessed within and between person **Young adults** (n=191)	✓ The perception of how harmful vaping is decreased within each condition: positive (univalent ppts mean difference [MD] = -0.76, 95% CI = -1.18 - -0.33; ambivalent ppts mean difference = -0.94, -1.35 - -0.54), negative (univalent ppts MD = -0.54, -0.93 - -0.15; ambivalent ppts (MD = -0.97, -1.46 - -0.48), ambivalent (univalent ppts MD = -0.90, -1.29 - -0.51; ambivalent ppts (MD = -1.13, -1.60 - -0.66) but there was no	✓/× Perceptions of vaping benefits relative to smoking decreased in some, but not all, conditions (average of: a) you can use e-cigarettes in places where smoking is not allowed, b) people can use e-cigarettes without affecting those around them, c) e-cigarettes are a safer alternative to regular cigarettes, d) e-cigarettes are less toxic than ordinary cigarettes, e) using e-cigarettes is a good way to	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
		significant main effect of message condition. Attitudinal ambivalence towards vaping was treated as a moderator.	express your independence)/ Specifically, benefits relative to smoking decreased among univalent, but not ambivalent, ppts in the negative condition (univalent MD = -0.40, -0.68 - -0.13; ambivalent MD = -0.13, -0.47-0.21) and ambivalent condition (univalent MD = -0.32, -0.60 - -0.04; ambivalent MD = 0.21, -0.12-0.54). In the positive condition, perceived benefits of vaping did not change in either group (univalent MD = -0.19, -0.49-0.10; ambivalent MD = 0.10, -0.18-0.39). There was no significant main effect of message	

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
			condition. Attitudinal ambivalence towards vaping was treated as a moderator.	
Noar et al. (2019) (9) US	**Written information about vaping harms and addictiveness.** Participants viewed 1 of 3 messages, the order of which was randomised: 1) nicotine ("e-cigarettes and vaping devices contain nicotine. Nicotine is an addictive chemical.") 2) chemical ("the liquid in e-cigarettes and vaping devices contains harmful chemicals. Poisonous if swallowed.") 3) brain ("nicotine in e-cigarettes and vaping devices may harm teen brain development"). **Youth** (n=61) **One-group experiment** with change assessed within person	✓ The following perceptions increased from pre- to post-exposure: perceived dangers of vaping (mean=1.70 to 2.56, p<.001), worry about vaping risks (mean=3.52 to 3.79, p=.048), perceiving that vaping would have health consequences (mean=3.32 to 3.70, p<.001), perceiving that vaping would lead to addiction (mean=2.60 to 2.89, p=.021), perceiving that e-cigarettes contain harmful chemicals (66% to	✓ Perceiving that vaping is equally/more harmful than smoking increased from pre- to post-exposure: (31% to 51%, p=.004).	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
		89%, p<.001), perceiving that vaping can harm teen brain development (53% to 80%, p<.001). Perception that e-cigarettes contain addictive nicotine did not increase significantly from pre- to post-exposure (82% to 92%, p=.110).		
Pepper et al. (2019) (10) US	**Written information about uncertainty of vaping harms.** Participants were randomised to view an uncertainty message (highlighting uncertainty about the harms of vaping) or a control message. The uncertainty message highlighted uncertainty about the harms of vaping, e.g., “Not enough scientific evidence exists to say for sure how using electronic vaping products could affect your health in the short or long term”. The control message concluded “Studies have shown that the liquids in vaping products contain chemicals that are harmful when inhaled.” **Adults** (overall n=2508; n=1253 uncertainty condition, n=1255 control condition)	✓ Respondents who viewed the uncertainty message had lower ratings of perceived harm (“How harmful do you believe using electronic vaping products is to your health?") than those who viewed the control message in unadjusted analyses (B = -0.15, 95% CI = -0.24 to -0.05, p<.01) and when adjusting	N/A	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
	Randomised experiment with change assessed within and between person Adjusted for smoking, vaping, the interaction of smoking and vaping, demographics, and health literacy.	for sample characteristics (B = -0.13, -0.22 to -0.04, p<.01).		
Yang & Popova (2020) (11) US	**Written information about reduced harm of vaping relative to smoking and nicotine.** Participants were randomised to 1 of 4 conditions: 1) exposure to comparative risk messages 2) exposure to comparative risk messages + FDA nicotine addiction warning 3) exposure to comparative risk messages + nicotine fact sheet 4) control (exposure to bottled water advertisements) **Adult current/recent former smokers** (n=1528) **Randomised experiment** with change assessed within and between person Adjusted for gender, age, race, education, daily smoking, vaping status, smoking identity.	N/A	✓ Perceiving vaping is less harmful than smoking increased after exposure to all three comparative risk messages, compared with the control condition (43.9% to 45.8%): Comparative risk message alone (41.6% to 55.9%; AOR=2.28, 1.36-3.82, p<.001) Comparative risk + FDA nicotine warning (41.8% to 54.6%; AOR=1.86, 1.12-3.12, p<.001) Comparative risk + nicotine fact sheet (37.2% to 53.9%; AOR=2.40, 1.43-4.03, p<.001).	✓/× Disagreeing with the false statement that nicotine is the main cause of smoking-related health problems increased to a greater extent in the comparative risk condition with a nicotine fact sheet (15.2% to 24.1%) than other conditions: comparative risk alone (17.9% to 15.1%; AOR=3.84,

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
			The 3 comparative risk message conditions did not differ from each other.	1.94-7.59, p<.001), comparative risk + FDA warning (17.2% to 15.9%; AOR=2.95, 1.54-5.68, p<.001), control (15.3% to 12.7%; AOR=4.15, 2.06-8.38, p<.001). There were no differences between the 4 message conditions on perceiving that nicotine is the main addictive substance in tobacco, compared with the control condition (85.7%): comparative

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
				risk messages alone (82.6%; AOR=0.79, 0.42-1.47) 2) comparative risk messages + FDA warning (82.8%; AOR=0.92, 0.49-1.71), comparative risk messages + nicotine fact sheet (84.8%; AOR=0.93, 0.49-1.76). The proportion of participants who perceived that nicotine is the main addictive substance in tobacco ranged from 78.9% to 83.0% at baseline.

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Yang et al. (2018) (12) US	**Written information about reduced harm of vaping relative to smoking.** All participants were shown 3 written statements: 1 message targeting their group [targeted message] and 2 messages targeting other groups [nontargeted messages]) in a random order. For example, the targeted message for Older Freedom Smokers included statements such as "You are in charge and you call the shots about how you want to live your life. One choice you've made is to smoke cigarettes… now's the time to quit and get your freedom back. Some smokers say using ENDS helped them quit combusted ones. There is research that says ENDS may be as effective as the nicotine patch for helping people quit smoking." **Adult current smokers** (overall n=580, n=180 Older Freedom Smokers (OFS), n=200 Reluctant Smokers (RS), n=200 Young Enthusiasts (YE)) **Non-randomised experiment** with changes assessed within and between person Adjusted for perceived absolute smoking harm, response efficacy of vaping to help quit smoking, and response efficacy of vaping to reduce smoking	✓/× The perception that vaping is harmful increased to a greater extent after exposure to targeted vs. nontargeted messages among some, but not all, groups. Specifically, increases were seen among Young Enthusiasts ($F(1,192)=10.76$, $p<0.01$, $\eta p2=0.05$), but not Reluctant Smokers or Older Freedom Smokers (contrasts not reported).	✓/× The perception that vaping is less harmful than smoking increased to a greater extent after exposure to targeted vs. nontargeted messages among some, but not all, groups. Specifically, increases were seen among Reluctant Smokers (targeted vs. Older Freedom Smoker message: AOR=2.44, 1.01-5.89, p<.05; targeted vs. Young Enthusiast message: AOR=2.74, 1.08-6.94, p<.05), but not Older Freedom Smokers (targeted vs. Reluctant Smoker message: AOR=1.38, 0.46-	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
			4.12, p>.05; targeted vs. Young Enthusiast message: AOR=1.87, 0.65-5.37, p>.05) or Young Enthusiasts (targeted vs. Older Freedom Smoker message: AOR=0.80, 0.26-2.43, p>.05; targeted vs. Reluctant Smoker message: AOR=0.37, 0.11-1.23, p>.05).	
Yang et al. (2019) (13) US	**Written information about reduced harm of vaping relative to smoking.** Participants were randomised to 1 of 3 conditions: 1) exposure to comparative risk messages (stated that switching to e-cigarettes can reduce health risks, used positive images and lighter colours) 2) exposure to negative comparative risk messages (highlighted the dangers of smoking, used threat-based images and negative colours, and also stated that switching to e-cigarettes from smoking	N/A	✓ Perception that vaping is less harmful than smoking increased after exposure to reduced risk messages (conditions 1 and 2 combined), to a greater extent than the control condition (OR=1.29, 1.12-	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
	completely can reduce health risks) 3) control (exposure to bottled water advertisements). **Adult current/recent former smokers** (n=1400) **Randomised experiment** with change assessed within and between person Adjusted for sex, age, race, education level, response efficacy, self-efficacy, daily smoking, vaping status, last year quit attempt, smoking identity.		1.48, p<.001). Conditions 1 and 2 did not differ in changing perceptions that vaping is less harmful than smoking (OR=1.13, 0.97-1.31, p>.05).	
Yang et al. (2020) (14) US	**Written information about nicotine.** Participants were randomised to 1 of 2 conditions: 1) exposed to a nicotine fact sheet (fact sheet highlighting that nicotine is not the main cause of harm from smoking, but that nicotine is a poison at very high doses, is not safe to use in pregnancy, can harm the adolescent brain, and is addictive) 2) control (exposed to bottled water advertisements) **Text statements** (nicotine fact sheet) **Adult current/recent former smokers** (n=756) **Randomised experiment** with change assessed within and between person. Adjusted for gender, age, race, education, daily smoking, vaping status, past year quit attempt, smoking identity.	N/A	**×** No difference pre- to post-exposure in accurately perceiving vaping is less harmful than smoking in the nicotine fact sheet (44.2% to 45.0%) vs. control (43.9 to 42.3%) conditions (unadjusted RR=1.06, 0.90-1.25, p>.05; adjusted RR=1.07, 0.95-1.20, p>.05).	**✓/×** After viewing the nicotine fact sheet, the proportion of adult current/recent former smokers holding accurate nicotine perceptions increased (12.7% to 26.2%) to a greater extent than in the control condition

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
				(15.3% to 12.7%) (unadjusted RR=2.06, 95% CI=1.51-2.82, p < 0.001; adjusted RR=2.29, 1.76-2.98, p<.001). However, there was no difference pre- to post-exposure in perceived addictiveness of nicotine in the nicotine fact sheet (83.9% to 82.3%) vs. control (83.3% to 85.7%) conditions (unadjusted RR=0.96, 0.90-1.02), p>.05; adjusted RR=0.96, 0.91-1.02, p>.05).

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Yang et al. (2021) (15) US	**Written information about reduced harm of vaping relative to smoking.** Participants were randomised to 1 of 2 conditions: 1) exposure to a reduced risk message (which stated that switching completely to e-cigarettes could reduce smokers' risks for smoking-related diseases, and if smokers cannot quit smoking, they can instead switch completely to e-cigarettes) + an FDA nicotine warning label ("This product contains nicotine. Nicotine is an addictive chemical") 2) control (exposure to bottled water advertisements). **Adult current/recent former smokers** (n=761) **Randomised experiment** with change assessed within and between person Adjusted for gender, race, age, education, nicotine dependence, past quitting attempt, current/ever vaping, ever switch to a lower tar or nicotine cigarette.	N/A	✓ Accurate perceptions that switching from smoking to vaping would reduce health risks (composite measure of agreement with three items: "Switching completely to e-cigarettes is effective at reducing my chances of getting cancer", "If I switch completely to e-cigarettes, I am less likely to get a serious disease", "If I switch completely to e-cigarettes, I will have fewer health risks") increased after exposure to a comparative risk message and nicotine warning label compared with the control condition	N/A

Authors and country	Methods: Description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: Measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
			(b=0.12, p=.002, η2= 0.005, r=0.07). Findings differed amongst current/recent former smokers who had high vs. low efficacy beliefs, such that perceptions of reduced health risks relative to smoking only increased among those with low (b=0.25, p<.001), but not high (b=0.01, p=.910), efficacy beliefs.	

✓ = a statistically significant effect of the intervention on the outcome.

× = no statistically significant effect of the intervention on the outcome.

✓/× = some statistically significant effects of the intervention on the outcome (for example, for some sample subgroups but not others, or for some measures of vaping harms but not others).

Table 8. Associations between interventions involving education about vaping and changes in vaping harm perceptions (research question 1)

Authors and country	Methods: description of intervention, sample description, study design, follow-up covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Baer et al. (2021) (16) US	**Educational workshop** providing information about smoking and vaping among youth and associated health outcomes (e.g. heart disease, cancers and respiratory diseases). **Adult school educators** (n=41) **One-group experiment** with change assessed within person	**×** Number of "correct" responses to health conditions that research has shown to be affected by vaping (2.4% [n=1] to 31.7% [n=13], p=.32) or that research suggests could be affected by vaping (85.4% [N=35] to 82.9% [n=34], p=.05) did not significantly change after the workshop. Note that this study did not define what "correct" responses were.	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Gaiha et al. (2021) (17) US	**Educational workshop**. Brief educational presentation on vaping, which included information about e-cigarette types, contents, health effects and nicotine addiction and the tobacco industry's manipulation of youth through advertising and marketing and flavours. **Youth** (n=2889, although the analyses reported here range from n=2613 to n=2706) **One-group experiment** with change assessed within person	✓ The following perceptions increased after the workshop: agreement that e-cigarette smoke is not harmless water vapor (90.7% to 93.7%, p<.001), agreement that the specific ingredients in pod-based systems and their long-term effects are not known (63.2% to 65.9%, p=.008), and agreement that e-cigarettes are addictive (84.6% to 88.5, p<.001)	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Little et al. (2016) (18) US	**Educational workshop**. Brief tobacco intervention involving interactive group discussions with 5 intervention targets: (1) enhancing perceived behavioral control (2) correcting subjective norms of tobacco/nicotine product use among Airmen (3) fostering negative attitudes towards tobacco/nicotine product use through peer led discussions (4) increasing knowledge* regarding the health consequences of tobacco/nicotine product use (5) delivering the brief tobacco intervention using a motivational interviewing style. *Note that there is no definition in the paper of 'knowledge' **Young adult military personnel** (n=1055) **One-group experiment** with change assessed within person	✓ The perception of how harmful vaping is to your health (1 ["Not harmful to your health"] to 7 ["Extremely harmful to your health"]) increased after the workshop among both users (mean=3.89 [SD=2.0] to 5.68 [SD=1.7]) and nonusers (mean=4.75 [SD=2.0] to 6.18 [SD=1.4]) of tobacco/nicotine products (all p<.0001). The proportion of "Don't know" responses also decreased from after the workshop (9.9% to 4.2%, p<.05).	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Sergakis et al. (2019) (19) US	**Educational video**. Participants viewed a 7 minute educational video which: 1) informed students of current research about the health effects of vaping 2) illustrated vaping prevalence, and 3) addressed common vaping misperceptions Note that there is no definition in the paper of 'current research about the health effects of e-cigarettes', 'e-cigarette prevalence', or 'misperceptions'. **Adults, university students** (n=115) **One-group experiment** with change assessed within person	N/A	✓ Perceiving that vaping will help to decrease the health problems caused by smoking (1 ["strongly disagree"] to 7 ["strongly agree"]) decreased after the educational video (median=4, IQR=2-5, to median=2, IQR=1-4, p<.001).	N/A

✓ = a statistically significant effect of the intervention on the outcome.

× = no statistically significant effect of the intervention on the outcome.

✓/× = some statistically significant effects of the intervention on the outcome (for example, for some sample subgroups but not others, or for some measures of vaping harms but not others).

Table 9. Associations between interventions involving mass media campaigns or advertisements and changes in vaping harm perceptions (research question 1)

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Booth et al. (2019) (20) UK and US	**Advertisements**. Participants were randomised to view 1 of 15 advertisements online. Advertisements included the following themes: e-cigarettes as a smoking cessation tool, healthier than (tobacco) cigarettes, aesthetically pleasing, celebrity endorsed, sporty, an alternative to cigarettes in places where cigarettes were restricted, as satisfying, cheaper, more fragrant and as cool as cigarettes. Advertisements were chosen from a pool of 200 different advertisements displayed online between 2013 and 2016. **Adults** (n=765) **Randomised experiment** with change assessed within person.	✓ Perception of vaping as healthy (1 [“strongly disagree”] to 7 [“strongly agree”]) increased after viewing advertisements among non-smokers (M=2.5 [SD=1.4] to M=2.7 [SD=1.5]; Z=2.97, p=.003), smokers (M=3.0 [SD=1.5] to M=3.2 [SD=1.5]; Z=2.21, p=.027), and dual users (M=3.8 [SD=1.3] to M=4.0 [SD=1.4]; Z=2.53, p=.011) but not among vapers (M=4.8 [SD=1.6] to M=4.8 [SD=1.6]; p>.05).	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
England et al. (2021) (21) US	**Mass media campaign**. Participants were randomised to view stills of videos from the "Rethink Vape" campaign (rethinkvape.org) – which highlights vaping risks, including that vaping is not less harmful than smoking and risks of developing specific diseases, and risks of nicotine addiction – or a control (Rev Your Bev, which educates about sugar content in popular drinks) **Youth** (n=268) **Randomised experiment** with change assessed within and between person.	✓ Perception that vaping is harmful (composite of 4 items, e.g., that vaping seriously harms health) increased after exposure to the intervention (M=3.8 [SD=1.0] to M=4.2 [SD=0.7], p<.05), but not control (M=3.9 [SD=0.9] to M=4.0 [SD=0.9], p>.05) campaign (F(266)=35.62, p<.001). "Vaping knowledge" (assessed using 15 items e.g., "Flavoring chemicals found in vaping devices have been linked to a serious lung disease") also increased after exposure to the intervention (M=3.4 [SD=0.6] to M=3.8 [SD=0.5], p<.05) but not the control (M=3.5 [SD=0.5] to M=3.7 [SD=0.6], p>.05) campaign (F(266)=59.19, p<.001). The campaign led to significant change in the same direction regardless of nicotine/tobacco user status.	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Ratneswaran et al. (2019) (22) UK	**Advertisements**. Participants were exposed to 5 images of recent e-cigarette advertisements (e.g., “a healthier option to smoking”, “beating the smoking ban laws”, “no need to quit smoking,” “experience the breakthrough: no tobacco, no smoke, just pure satisfaction for smokers,” “cigarettes, you’ve met your match”). **Young adults** (n=106) **One-group experiment** with change assessed within person	× Mean scores on the item “E-cigarettes are harmful to health” (response options not stated) did not change significantly after the intervention overall (mean=2.09 [SD=0.8] to mean=2.25 [SD=1.0], χ^2=0.15, p=.105) or when split by smoker/vaper subgroups: Smokers (mean=1.9 [SD=0.9] to 2.2 [1.1], χ^2=0.26, p=.150) Non-smokers (mean=2.3 [SD=0.8] to 2.3 [0.8], χ^2=0.05, p=.568) Vapers (mean=2.4 [SD=1.0] to 2.5 [1.07], χ^2=0.12, p=.942) Non-vapers (mean=2.1 [SD=0.8] to 2.2 [0.9], χ^2=0.18, p=.050)		

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Tan et al. (2015) (23) US	**Self-reported exposure to information about vaping**. Participants self-reported their exposure to a) incorrect information about vaping, and b) refuting incorrect information about vaping (how often they had heard that each claim is FALSE), across 5 items (averaged): 1) E-cigarettes have been shown to cause lung cancer 2) E-cigarettes are just as dangerous as actual cigarettes for health 3) The nicotine delivered by e-cigarettes may still contribute to heart disease 4) Vapors from e-cigarettes contain tar 5) Electronic cigarettes contain carcinogenic chemicals that make some as harmful as normal tobacco. Items for exposure to incorrect information were averaged, and items for exposure to refuting information were averaged. **Young adults** (n=411) **Cohort study (survey)** with 3-month follow-up and changes assessed within person. Adjusted for age, gender, race, ethnicity, health status, household income, medical coverage, having a family member or close friend with history of cancer, smoking status, interest in the respective health topics.	**✓/×** Self-reported exposure to refuting incorrect information about vaping (Beta = -0.11, 95% CI = -0.20, -0.01, p<.005), but not exposure to incorrect information about vaping (Beta = 0.06, -0.05, 0.17), predicted a reduction in a composite measure of vaping harm perceptions at follow-up. Perceptions were measured as changes in agreement with items 1 through 5 listed on the column to the left, while adjusting for baseline perceptions (relative harm, absolute harm, risk of specific diseases).		

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Tattan-Birch et al. (2020) (24) UK	**Mass media campaign** in Greater Manchester, by CRUK, which highlighted that vaping is less harmful than smoking, nicotine is not responsible for most of the health harms from smoking, and many people quit smoking using e-cigarettes. Campaign was via adverts on buses, billboards etc., press coverage, and Facebook. The control group was participants from Yorkshire & Humber and the North East of England, where no campaign was implemented. **Adults** (n=1637) **Repeated cross-sectional survey** with 2-3 month follow-up. Adjusted for age group, sex, social grade	N/A	**×** Insufficient evidence to determine whether the campaign affected the relative perception that vaping is less harmful than smoking: campaign region change (55.0% to 57.8%) vs. control region change: (48.3% to 57.5%) (OR=0.76, 0.51-1.13, p=.18, Bayes Factor = 0.36).	N/A

✓ = a statistically significant effect of the intervention on the outcome.

× = no statistically significant effect of the intervention on the outcome.

✓/× = some statistically significant effects of the intervention on the outcome (for example, for some sample subgroups but not others, or for some measures of vaping harms but not others).

Table 10. Associations between interventions involving packaging/warning labels and changes in vaping harm perceptions (research question 1)

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Kimber et al. (2020) (25) UK	**Packaging/warning labels**. Participants were randomised to view images of 1 of 6 messages on e-cigarette packs: TPD1 (TPD health warning as per currently implemented in the UK: "This product contains nicotine which is a highly addictive substance") TPD2 (TPD longer health warning as currently implemented in many EU countries: "This product contains nicotine which is a highly addictive substance. It is not recommended for non-smokers") COMP ("Use of this product is much less harmful than smoking") TPD1+COMP ("This product contains nicotine which is a highly addictive substance. Use of this product is much less harmful than smoking") TPD2+COMP ("This product contains nicotine which is a highly addictive substance. It is not recommended for non-smokers. Use of this product is much less harmful than smoking" Control: No message (no message condition using the same e-cigarette pack images) **Adult non-current vapers** (n=2495) **Randomised experiment** with change assessed within and between person Adjusted for motivation to quit smoking and cigarette dependence.	✓ Perception of vaping harmfulness ('How harmful do you think e-cigarettes are?' from 1 = Not at all to 7 = Extremely) increased following exposure to the TPD messages (mean=5.23 [CI: 5.19–5.27]) compared with the TPD absence conditions (mean=5.01 [4.95–5.07]). Perceptions of vaping harmfulness decreased in both smokers and non-smokers following exposure to COMP alone (smokers: mean=4.55 [4.41–4.69], non-smokers: mean=5.45 [5.33–5.56]) (statistics not reported). However, when compared with no message, reduction in perceptions of vaping harm was shown after exposure to the COMP alone only in smokers. Perceptions of vaping addictiveness ('How addictive do you think e-cigarettes are?' 1	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
		= Not at all to 7 = Extremely) increased in both smokers and non-smokers following exposure to the TPD relative to those exposed to no messages and those exposed to the COMP. Perceptions of vaping addictiveness decreased in both smokers and non-smokers following exposure to the COMP message compared to those exposed to both the TPD+COMP messages.		
Lee et al. (2018) (26) US	**Packaging/warning labels**. Participants were randomised to view an FDA warning label ("WARNING: This product contains nicotine derived from tobacco. Nicotine is an addictive chemical.") or an e-cigarette company warning label ("This product is not a smoking cessation product and has not been tested as such. This product is intended for use by persons of legal age or older, and not by children, women who are pregnant or breastfeeding, or persons with or at risk of heart disease, high blood pressure, diabetes, or taking medicine for depression or asthma. Nicotine is addictive and habit forming, and it is very toxic by inhalation, in contact with the skin, or if swallowed. Nicotine can increase your heart rate and blood pressure and cause	**✓/×** Perceived risk of vaping (sum of items: die prematurely, damage overall health, damage lungs, and develop: lung cancer, heart disease, mouth/teeth problem, nicotine addiction) increased after exposure to the FDA label (beta = 0.10, SE = 0.05, p<.05) but not the e-cigarette company label (beta = 0.06, SE = 0.04, p>.05).	**×** Perceived advantage of vaping relative to smoking (sum of items: e-cigarettes are less harmful than traditional cigarettes, can help reduce tobacco consumption can help quit smoking, are less addictive than traditional cigarettes) did not change after either the FDA label (beta=-0.03, SE=0.06, p>.05) nor the e-cigarette company label (beta=-0.01, SE=0.05, p>.05).	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
	dizziness, nausea, and stomach pain. Inhalation of this product may aggravate existing respiratory conditions. Ingestion of the non-vaporized concentrated ingredients in the cartridges can be poisonous.") **Young adults** (overall n=666: n=338 FDA condition, n=328 e-cigarette company condition) **Randomised experiment** with change assessed within and between person Adjusted for sex, race/ethnicity, age, tried vaping, e-cigarette knowledge.			
Popova & Ling (2014) (27) US	**Packaging/warning labels**. Participants were randomised to view 1 of 5 warning labels or a control: 1) current warning label ("Warning: This product is not a safe alternative to cigarettes") 2) graphic warning label (picture of a mouth sore and words "Warning: This product can cause mouth cancer") 3) R. J. Reynolds's proposed "lower risk" label ("Warning: No tobacco product is safe, but this product presents substantially lower risks to health than cigarettes") 4) "FDA Approved" label 5) an advertisement for a tobacco product with no warning label 6) control: advertisements for a non-tobacco consumer product (e.g., cell phone or gum). **Adult non-established smokers or vapers** (overall n=483: n=75 no warning label, n=74	✓/× Perceived harm of vaping (average of two items: "In your opinion, how harmful is e-cigarettes to general health?" and "In your opinion, to what extent does e-cigarettes cause cancer?") increased after viewing the current warning label (mean=5.51 to 5.96, d=0.24, p<.05) and graphic warning label (mean=4.33 to 5.67, d=0.54, p<.05) but not the 'lower risks' label (mean=4.81 to 5.15, d = 0.15, p>.05), 'FDA-approved' label (mean=5.15 to 5.24, d=0.04, p>.05), no label (mean=5.08 to 5.20, d=0.05, p>.05), or control (mean=4.56	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
	current warning label, n=75 lower risk warning label, n=79 FDA warning label, n=76 graphic warning label, n=76 control warning label) **Randomised experiment** with change assessed within and between person	to 4.94, d=0.19, p>.05). There was a significant time*group interaction for the effects of warning labels on changes in perceived harm (F(5, 474) = 3.38, p < .01).		

✓ = a statistically significant effect of the intervention on the outcome.

× = no statistically significant effect of the intervention on the outcome.

✓/× = some statistically significant effects of the intervention on the outcome (for example, for some sample subgroups but not others, or for some measures of vaping harms but not others).

Table 11. Associations between interventions involving video games and changes in vaping harm perceptions (research question 1)

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Hieftje et al. (2021) (28) US	**Video game** focused on tobacco use prevention in adolescents, which teaches teens about peer pressure and the use of tobacco products (including e-cigarettes) and on "correcting participants' misperceptions and misinformation around use of tobacco and vaping products." **Youth** (n=560) **One-group experiment** with change assessed within person Note that there is no definition in the paper of 'misperceptions'	✓ Perception of vaping harms and addictiveness increased after playing the video game. Specifically, the proportion of youth who responded 'yes' to the following statements increased after playing the video game: "Do you think e-cigarettes are dangerous?" 88.8% to 94.6% (p<.0001), "Do you think smoking e-cigarettes is harmful to your health?" 88.4% to 94.3% (p<.0001), "Once a teen has started using e-cigarettes, do you think it would be difficult for them to quit?" 83.4% to 91.8% (p<.0001), "How likely is a teen to become addicted to e-cigarettes?" 72.0% to 85.0% (p<.0001).	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Pentz et al. (2019) (29) US	**Video game** (smokeSCREEN), in which players work on specific skills that could transfer to the real world to help them avoid risky behaviours, such as refusing offers by peers to use e-cigarettes, encountering a character who says e-cigarettes are safe, or should be tried because of a great flavour. Exposure took place over 4 hours over 4 weeks. **Youth** (n=80, of which n=14 were included in analyses of the association between playing the video game and changes in vaping harm perceptions) **One-group experiment** with change assessed within person	✓ Perceived harms from vaping increased after playing the video game. Specifically, mean scores on the item “How much do you think people harm themselves when they use e-cigarettes” (1=no harm, to 4=a lot) increased from before (3.25 [SD=0.36]) to after (3.40 [SD=0.40]) playing the video game (t=3.41, p=.001).	N/A	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Weser et al. (2021) (30) US	**Video game** (virtual Reality, VR) that teaches adolescents about the health risks of vaping e-cigarettes while providing a virtual environment for adolescents to practice refusing peer pressure to vape e-cigarettes. **Youth** (n=47) **One-group experiment** with change assessed within person	✓/× Perceived harm from vaping but not perceived addiction or short-term safety, increased after playing the video game. Perceived harm from vaping (composite of 3 items, e.g., "How much do you think people harm themselves when they breathe in other people's e-cigarette or JUUL vapor") increased from before (mean=2.84 [SD=0.76]) to after (mean=3.25 [SD=0.74]) playing the video game (t(34)=-3.37, p=.002, d=0.53). Perceiving It is hard to get addicted to vaping/JUULing showed no significant increase from before (mean=1.11 [SD=1.36] to after (mean=1.82 [SD=1.34]) playing the video game (d=0.45). Perceived ease of quitting vaping/JUULing showed no significant increase from before (mean=2.89 [SD=0.83]) to after (mean=3.03 [SD=0.79]) playing the video game (d=0.13). Perceiving it is safe to JUUL/vape for a year as long as you quit after showed no significant increase from before (mean=1.65 [SD=0.89]) to after (mean=1.53 [SD=0.71]) playing the video game (d=0.12)	N/A	N/A

✓ = a statistically significant effect of the intervention on the outcome.

× = no statistically significant effect of the intervention on the outcome.

✓/× = some statistically significant effects of the intervention on the outcome (for example, for some sample subgroups but not others, or for some measures of vaping harms but not others).

Table 12. Associations between EVALI and changes in vaping harm perceptions (research question 1)

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
Alber et al. (2021) (31) US	Evaluated the impact of EVALI at the population level **Adults** (n=1057) **Repeated cross-sectional survey** with 3 month follow-up Adjusted for age, sex, race/ethnicity, income, education, vaping, smoking.	✓ Positive harm perceptions of vaping (a composite of absolute and relative perceptions) decreased from pre- to post- EVALI (b=-.066, SE=.067, p<.05). Perceptions were measured using 10 items (each scored from 1-7 and averaged; higher scores indicate more positive perceptions): E-cigarettes are much less harmful than traditional cigarettes; People risk harming other people if they use e-cigarettes; E-cigarettes are harmful for one's short-term health; E-cigarettes are harmful for one's long-term health; E-cigarettes do not contain any of the toxic chemicals that can be found in traditional cigarettes; E-cigarettes are bad; E-cigarettes are harmful to the environment; I like e-cigarettes; I like the flavors of e-cigarettes; E-cigarettes help people to quit smoking traditional cigarettes.		N/A
Morgan et al. (2021) (32) US	Evaluated the impact of EVALI at the population level **Adult current/former smokers** (n=1209) **Repeated cross-sectional survey** with 1 month follow-up	✓/× Some, but not all, measures of perception of vaping harms increased after EVALI. The perception of how harmful e-cigarettes are to your health, (1 = not at all harmful to 4 = extremely harmful) increased from mean (M)=2.67 (SD=0.9) to M=2.90 (SD=1.0) (Hedge's g=-.25, p<.001). Change was driven by ever vapers, specifically former vapers (p<.01) and current vapers	✓ Perceived risk of e-cigarette use and lung damage (both on a scale of strongly disagree (1) to strongly agree (5)) increased from before to after EVALI when risks were compared to cigarette smoking were considered (p < .05).*	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
		(p<.05). Never vapers' perceptions trended in the same with a smaller and non-significant change (p=.051). However, the perceived risk of e-cigarette use and lung damage (both from 1 = strongly disagree to 5 = strongly agree) did not change (p>.05).* *EVALI therefore affected beliefs about cigarettes, but only when a comparison to cigarettes was invoked. Differences only occurred in never and former vapers (p<.05), but not current vapers (p>.05).		
Moustafa et al. (2021) (33) US	Evaluated the impact of EVALI at the population level **Youth** (n=1539) **Cohort study (survey)** with 6 month follow-up and changes assessed within person Adjusted for past 30-day nicotine vaping, past 30-day marijuana vaping, sex, race, ethnicity, peer vaping acceptance, sensation seeking, past 6-month		✓ Perception that vaping is less harmful to others and less addictive than smoking decreased after EVALI. Specifically, perceived risks (mean of two items from 0 [strongly disagree] to 3 [strongly agree]: 1) E-cigarettes might be less harmful for people to be around than cigarettes, 2) E-cigarettes	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
	smoking.		might be less addictive than cigarettes) decreased from before to after EVALI (unadjusted B=-0.15,-0.22 to -0.07; adjusted B=-0.14, -0.22 to -0.06).	
Tattan-Birch et al. (2020) (54) UK	Evaluated the impact of EVALI at the population level **Adult current smokers** (n=3215) **Repeated cross-sectional survey** with 48 month follow-up Adjusted for sex, age, social grade, race/ethnicity, vaping.		✓ Accurate perception that vaping is less harmful than smoking decreased after EVALI, from 37.0% to 30.9% (RR=0.83, 95% CI=0.76-0.92, p<.001; ARR=0.81, 0.74-0.90, p<.001). Fewer smokers reported not knowing which product was more harmful (10.4% to 8.1%; RR=0.78, 0.63-0.98, p=.03; ARR=0.78, 0.62-0.97, p=.03). The proportion of individuals who perceived e-cigarettes as equally harmful than smoking	N/A

Authors and country	Methods: description of intervention, sample description, study design, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with intervention		
		Absolute vaping harm	Relative vaping harm	Nicotine risk
			increased (39.9% to 43.8%; RR=1.10, 1.01-1.19, p=.01; ARR=1.09, 1.01-1.18, p=.02) The proportion of individuals who perceived e-cigarettes as more harmful than smoking increased (12.7% vs. 17.2%; RR=1.36, 1.15-1.61, p<.001; ARR=1.38, 1.17-1.62, <.001).	

✓ = a statistically significant effect of the intervention on the outcome.

× = no statistically significant effect of the intervention on the outcome.

✓/× = some statistically significant effects of the intervention on the outcome (for example, for some sample subgroups but not others, or for some measures of vaping harms but not others).

Research question 2: to what extent are vaping harm perceptions predictive of any changes in vaping and smoking behaviours?

Study characteristics

We identified 21 studies that met our inclusion criteria for RQ2 (33-53). Study characteristics are shown in table 13.

Seventeen studies were from the US (33, 34, 36, 38-43, 45-51, 53), 2 were from the UK (35, 37), one from Canada (44), and one from the UK and Australia (52).

One study was funded by a tobacco company (Imperial Brands Plc. (45)).

Eleven studies took place in household settings (36, 37, 39, 41, 42, 45, 47-50, 53), 7 in online settings (34, 35, 38, 40, 44, 46, 51), one in online and telephone settings (52), one in hospital settings (43), and one in school settings (33).

Sample sizes ranged from 137 (44) to 21,693 (45). Seven studies were among adults (age 18+) (35, 41-43, 45, 49, 52), with 6 among young adults (age range 15-34, or mean age <25 years) (34, 37, 39, 40, 44, 51) and 8 among youth (age 12-17, or mean age <17 years, or school students) (33, 36, 38, 46-48, 50, 53). The proportion of males ranged from 37% (46) to 59% (35).

All studies were cohort studies (33-53).

Sixteen studies had a follow-up length of 12 months or less (33-37, 39, 41-45, 47-51). The maximum length of follow-up was 36 months (46, 52).

Risk of bias

Quality was low among all 21 studies (33-53), with scores on the adapted 5-star Newcastle Ottawa Scale ranging from one to 3 all indicating high risk of bias (≤3 stars indicates high risk of bias). All studies were considered to have risk of bias in terms of ascertainment of the exposure (harm perceptions) due to a lack of a validated measure for assessing vaping harm perceptions, and outcome (smoking/vaping) due to a lack of bio verification in all studies (Appendix 2).

Description of exposures and outcomes

Study exposures and outcomes are shown in table 14.

Studies could assess multiple exposures. Fourteen studies assessed perceived relative harms of vaping and smoking (33-35, 37, 39, 41-43, 45, 47-49, 51, 52). Ten studies assessed perceived absolute harm of harm from vaping (33, 36, 38, 40, 43, 44, 46, 48, 50, 53). Four studies assessed perceived absolute addictiveness of vaping (33, 40, 43, 50),

while one assessed perceived addictiveness of vaping relative to smoking (39). One study also assessed the perception that vaping can help smokers to quit smoking (39).

Studies could also assess multiple outcomes. Across all studies, 5 assessed both vaping and smoking behaviours as outcomes (36, 42, 46, 47, 49), 15 assessed vaping only (33-35, 37-41, 43, 44, 48, 50-53), and one – which was funded by a tobacco company – assessed smoking only (45). Among youth or young adults, 10 studies assessed vaping initiation (5 among youth: all never to ever (that is, trying) vaping (36, 38, 47, 48, 50), although 2 additionally assessed never to past 30-day vaping (38, 47)); 5 among young adults: 4 never to ever vaping (39, 40, 44, 46), one never to ever JUUL use and never to past 30-day JUUL use (51)); 3 of these studies also assessed smoking initiation, that is, never to ever (that is, trying) smoking, or never to past 30-day smoking (36, 46, 47)). Two studies assessed changes in past 30-day vaping among youth (33, 53) and one assessed changes in frequency of vaping among young adult smokers (34). One assessed vaping non-tobacco/menthol flavoured e-cigarettes among young adult vapers (37). One assessed both smoking and vaping escalation among young adult ever smoker/vapers (46). Among adults, 4 studies assessed vaping initiation: 2 never to ever vaping (35, 52), one non-vaper to vaper or remaining a vaper (41), one not currently vaping to currently vaping (42). Two assessed changes in frequency of vaping among adult smokers (43, 49) (one of which also assessed changes in frequency of smoking as well as transitions between vaping and smoking (49)), one assessed continued vaping (41), and one – funded by a tobacco company – assessed relapse to smoking among adult former smokers who vape (45).

Findings

We have grouped the findings into the following sections; whether vaping harm perceptions are predictive of changes in: vaping behaviours among youth and young adults, smoking behaviours among youth and young adults, vaping behaviours among adults, smoking behaviours among adults.

Whether vaping harm perceptions are predictive of changes in vaping behaviours among youth and young adults

Fourteen studies assessed associations between vaping harm perceptions and changes in vaping behaviours among youth and young adults (33, 34, 36-40, 44, 46-48, 50, 51, 53).

Of these 14 studies, 7 assessed associations between perceived relative harmfulness of vaping compared with smoking (sometimes including addictiveness (33)) and subsequent vaping behaviours among youth or young adults (33, 34, 37, 39, 47, 48, 51). Of these, all 7 found statistically significant associations between perceived harm of vaping relative to smoking and subsequent vaping behaviours (33, 34, 37, 39, 47, 48, 51). Specifically, perceiving less harm (and addictiveness (39)) from vaping relative to smoking was statistically significantly associated with subsequently trying vaping (that is, initiating ever

vaping) among baseline never vapers (39, 47, 48, 51). Perceiving less harm from vaping relative to smoking was also statistically significantly associated with an increase in number of days vaped among smokers, adjusting for baseline vaping (34), vaping non-tobacco/menthol flavoured e-cigarettes (vs. tobacco/menthol flavoured e-cigarettes) among vapers adjusting for smoking status (37), and an increase in past 30-day nicotine vaping (33) among a sample of predominant non-smokers and non-vapers at baseline. One of these studies (among young adult never vapers at baselines) also assessed perceived addictiveness of vaping relative to smoking, but found no statistically significant association with subsequently trying vaping (39).

Eight studies assessed associations between perceived absolute harm of vaping and vaping behaviours among youth or young adults (36, 38, 40, 44, 46, 48, 50, 53). Of these, all 8 found statistically significant inverse associations between perceiving vaping as harmful to health and: subsequently trying vaping among baseline never vapers (36, 38, 40, 44, 46, 48, 50), escalation of vaping among ever vapers (46), and increases in past 30-day vaping while adjusting for past 30-day vaping at baseline (53). However, for one of these studies that found a statistically significant association between perceived absolute harm of vaping (perceiving that people harm themselves when they vape) and subsequently trying vaping among baseline never vapers, temporality of the exposure and outcome was difficult to determine because harm perceptions were assessed as a change in perception between baseline and follow-up (36).

Two studies assessed associations with perceived risk of addiction from vaping among youth and young adults, and both found statistically significant associations between lower perceived addictiveness of vaping among baseline never vapers and subsequently trying vaping (40, 50).

One further study assessed the perception that vaping can help people quit smoking among young adults who had never vaped at baseline (either current, former or non-smokers), and found this perception was statistically significantly associated with subsequently trying vaping (39).

Whether vaping harm perceptions are predictive of changes in smoking behaviours among youth and young adults

Three studies assessed associations between vaping harm perceptions and smoking behaviours among youth (36, 46, 47). Of these 3, one assessed associations with perceived relative harm of vaping compared to smoking among baseline never smokers and never vapers and found no statistically significant association with subsequently trying smoking (47). Two studies assessed perceived absolute harm of vaping (for example, perceiving that people harm themselves when they vape) including perceived risk of addiction in one study (for example, developing specific health ailments and becoming addicted) and both found no statistically significant association with subsequently trying smoking among baseline never smokers (36, 46) or escalating smoking (that is, an

increase in the number of times smoked) among ever smokers (46). However, as mentioned above, for one of these studies that found no statistically significant association between perceived absolute harm of vaping (perceiving that people harm themselves when they vape) and subsequently trying smoking among baseline never smokers, temporality of the exposure and outcome was difficult to determine because harm perceptions were assessed as a change in perception between baseline and follow-up (36).

Whether vaping harm perceptions are predictive of changes in vaping behaviours among adults

Six studies assessed associations between vaping harm perceptions and vaping behaviours among adults (35, 41-43, 49, 52). Of these, all 6 assessed associations between perceived relative harmfulness of vaping compared with smoking and vaping behaviours among adults, including adult smokers/former smokers (35, 41-43, 49, 52). Of these 6 studies, 5 found statistically significant associations between perceived harm of vaping relative to smoking and subsequent vaping behaviours (35, 41, 42, 49, 52). Specifically, perceiving less harm from vaping relative to smoking was statistically significantly associated with: subsequently trying vaping among baseline never vapers who smoke/formerly smoked, or subsequently initiating current vaping among baseline non-current vapers who smoke (35, 42, 52) or subsequent initiation/continued vaping (41). In the other study, perceiving less harm from vaping relative to smoking increased the odds of concurrent past 30-day vapers and smokers switching to exclusive vaping, continued past 30-day vaping, but was not statistically significantly associated with changes in the number of days vaped or e-cigarette puffs on last day vaped (49). The sixth study found no statistically significant association between perceiving that vaping poses a risk to health as compared to smoking and changes in vaping frequency among current smokers (43).

Whether vaping harm perceptions are predictive of changes in smoking behaviours among adults

Three studies assessed associations between vaping harm perceptions and smoking behaviours among adult current (42, 49) or former (45) smokers. Of these, all 3 assessed associations between perceived relative harm of vaping compared with smoking and subsequent smoking behaviours (42, 45, 49), 2 of which found statistically significant associations (45, 49). Specifically, perceiving vaping as equally or more harmful than smoking was statistically significantly associated with subsequent relapse to smoking among former smokers in one study (funded by the tobacco industry) (45). In the other study of concurrent past 30-day smokers and past 30-day vapers, perceiving vaping as less harmful than smoking increased the odds of quitting smoking (that is, becoming an exclusive vaper) and reduced the odds of becoming an exclusive smoker (that is quitting vaping), increased the odds of remaining a dual smoker/vaper and was also statistically

significantly associated with an increase in the number of days smoked but not with changes in cigarettes per day (49). The third study found no statistically significant association between perceived relative harm of vaping and quitting smoking among current smokers (42).

Table 13. Study characteristics of articles that addressed research question 2: to what extent are vaping harm perceptions predictive of any changes in vaping and smoking behaviours? Studies are organised by outcome. All studies are cohort studies

Authors and year	Country, setting, and data collection period	Participants	Funding, conflicts of interest (COI)	Risk of bias (number of stars)[2]
Brikmanis et al. (2017) (34)	US, Online 03/2015 - 06/2016	n=348 Young adults (age 18-24 years, mean=20.5 years [SD=1.8]) 57% male 100% were monthly smokers for at least six months; 33% reported any vaping at baseline	NIDA COIs: NR	High (2)
Brose et al. (2015) (35)	UK, Online 11/2012-12/2014	n=1588 Adults (age 18+ years) 59% male, 41% female 100% past-year smokers (87% current, 13% former); 100% never tried vaping at baseline	UKCTAS, MRC, BHF, CRUK, ESRC, NIHR, SSA COIs: Author(s) have received a grant from Pfizer	High (3)
Chaffee & Cheng (2018) (36)	US, Household 09/2013 - 10/2015	n=8005 Youth (age 12-17 years; 65% 12-14 years, 35% 15-17 years) 50% male, 50% female 100% never used a tobacco product or e-cigarette at baseline	NIH, FDA COIs: None declared	High (3)

Authors and year	Country, setting, and data collection period	Participants	Funding, conflicts of interest (COI)	Risk of bias (number of stars)[2]
Chen et al. (2018) (37)	UK, Household 09/2013 - 10/2015	n=1421 Young adults (age 18-34 years; 42.5% 18-24 years, 57.5% 25-34 years)[1] 51% male, 49% female[1] 29% past month smokers, 30% ever but not past month smokers, 41% never smokers; 100% past month vapers at baseline	Department of Behavioral and Community Health, School of Public Health, University of Maryland College Park, Personalized Health Assessment Related to Medications COIs: None declared	High (3)
Chen-Sankey et al. (2019) (38)	US, Online 09/2013 - 10/2015	n=6983 Youth (age 12-17 years) Gender NR Never used a tobacco product or e-cigarette at baseline	National Institute on Minority Health and Health Disparities Division of Intramural Research, Yale TCORS COIs: None declared	High (3)
Choi & Forster (2014) (39)	US, Household (telephone) 10/2010 - 03/2012	n=1379 Young adults (mean age 24 years [SD=1.7] 48% male 18% current smokers, 13% former smokers, 69% non-smokers; 100% never vapers at baseline	NCI, NIH COIs: None declared	High (3)
Cooper et al. (2018) (40)	US, Online 11/2014 - 02/2015	n=2565 Young adults (age 18-25 years; mean age 20 years 66.4% female 5.1% current smokers; 100% never vapers at baseline	NCI, NIH, FDA COIs: None declared	High (3)

Authors and year	Country, setting, and data collection period	Participants	Funding, conflicts of interest (COI)	Risk of bias (number of stars)[2]
Elton-Marshall et al. (2020) (41)	US, Household 09/2013 - 10/2015	n=20628 Adults (age 18+) Gender NR 6.6% currently vaped at baseline, smoking status NR	NIDA, NIH, CDC, FDA COIs: Author(s) have received funding from Pfizer, received funding as an expert witness in litigation filed against the tobacco industry, and served as an expert for governments whose tobacco control policies have been challenged in litigation	High (3)
Harlow et al. (2019) (42)	US, Household 09/2013 - 10/2015	n=6592 Adults (age 18+) 56% male, 44% female 100% smoked at least 100 cigarettes in life and currently smoked; 100% not current vapers but 53% had tried vaping at baseline	NIH, CTP COIs: None declared	High (3)
Hendricks et al. (2018) (43)	US, Hospital 12/2012 and 10/2014	n=978 Adults (age 19-75 years, mean=45.5 years [SD=12.9]) 54.4% male 100% current smokers (smoked in past 30 days and identified as a current smoker by hospital admission record); 50% ever vaped, 21% vaped in the past 30 days	NIDA COIs: None declared	High (2)

Authors and year	Country, setting, and data collection period	Participants	Funding, conflicts of interest (COI)	Risk of bias (number of stars)[2]
Jayakumar et al. (2020) (44)	Canada, Online 02/2018 - 03/2019	n=137 Young adults (age 16-26 years, median=17-18 years depending on vaping status) 43% male, 57% female 92% never smoker, 5% non-current smoker, 0.1% current smoker; 100% never vaped at baseline	Ontario Ministry of Health and Long-term Care, Health System Research COIs: None declared	High (2)
Malt et al. (2020) (45)	US, Household 09/2013 - 10/2016	n=21693 Adults (age 18+ years) 48% male, 52% female at baseline Smoking and vaping status NR	Imperial Brands Plc. (tobacco industry) COIs: All authors are employees of Imperial Brands Plc.	High (2)
McKelvey et al. (2021) (46)	US, Online 07/2014 - 04/2019	n=772 Youth (mean age 16.0 years [SD=2.0]) 37% male, 63% female 13% ever smoked, 19% ever vaped at baseline	NCI, FDA CTP, National Heart, Lung, and Blood Institute, Stanford Maternal and Child Health Research Institute COIs: None declared	High (1)
Moustafa et al. (2021) (33)	US, High schools Spring and Fall 2019 (months not stated)	n=1539 Youth (mean age 16.7 years [SD=0.6]) 50% male, 50% female 3% smoked in the past 6 months, 97% did not; 11% vaped in the past 30 days, 89% did not at baseline	NCI COIs: None declared	High (3)

Authors and year	Country, setting, and data collection period	Participants	Funding, conflicts of interest (COI)	Risk of bias (number of stars)[2]
Nicksic et al. (2019) (47)	US, Household 09/2013-10/2015	n=5156 Youth (age 12-17 years) 53% male, 47% female 100% never smokers and never vapers at baseline	NIDA, NIH, NCI, FDA CTP, Virginia Foundation for Healthy Youth COIs: None declared	High (3)
Parker et al. (2018) (48)	US, Household 09/2013 - 10/2015	n=10081 Youth (age 12-17 years) 51.5% male, 48.5% female 100% never smokers and never vapers at baseline	NIH, FDA, TCORS Vulnerable Populations Working Group, NIDA, NCI, Center for Evaluation and Coordination of Training and Research in Tobacco Regulatory Science, National Institute of General Medical Sciences COIs: None declared	High (3)
Persoskie et al. (2019) (49)	US, Household 10/2014 - 10/2016	n=2211 Adults (age 18+) 52.5% male, 47.5% female 100% past 30-day smokers and past 30-day vapers	NIDA, NIH, FDA CTP, Department of Health and Human Services COIs: None declared	High (2)
Strong et al. (2019) (50)	US, Household and telephone 09/2013-10/2015	n=10081 Youth (age 12-17 years) Gender NR Smoking and vaping status NR	NIDA, NIH, CTP, FDA, Department of Health and Human Services COIs: Author(s) have received funding from Pfizer and received funding as an expert witness in litigation filed against the tobacco industry.	High (2)

Authors and year	Country, setting, and data collection period	Participants	Funding, conflicts of interest (COI)	Risk of bias (number of stars)[2]
Vallone et al. (2020) (51)	US, Online 02/2018 - 05/2019	n=12114 Young adults (age 15-34) 50% male, 50% female 48% never smokers, 37% former smokers, 14% current smokers; 6% current JUUL users, 14% ever JUUL users, 33% ever vapers, 12% current vapers	Truth Initiative COIs: None declared	High (3)
Yong et al. (2014) (52)	UK and Australia, Online and phone 07/2010 - 09/2013	n=1590 Adults (age 18+) Gender NR 100% current or former smokers; 100% never vaped at baseline	NCI, Robert Wood Johnson Foundation, CIHR, National Health and MRC of Australia, CRUK, Canadian Tobacco Control Research Initiative; Centre for Behavioural Research and Program Evaluation, NCI of Canada, CCS COIs: None declared	High (2)
Zheng et al. (2021) (53)	US, Household 10/2014 - 01/2018	n=6208 Youth (age 12-17 years) 51% male, 49% female Smoking status NR; 7% ever vaped, 93% never vaped at baseline	NIH, NDA COIs: None declared	High (3)

Notes: [1] Reported in whole sample, not subsample of past 30-day vapers included in analyses.

[2] Risk of bias was assessed for all studies using the Newcastle Ottawa Scale (NOS), with scores of >3 stars indicating low risk of bias and scores of ≤3 stars indicating high risk of bias.

BHF = British Heart Foundation, CCS = Canadian Cancer Society, CIHR = Canadian Institutes of Health Research, CRUK = Cancer Research UK, CTP = Center for Tobacco Products, ESRC = Economic and Social Research Council, FDA = Food and Drug

Administration, MRC = Medical Research Council, NCI = National Cancer Institute, NIDA = National Institute on Drug Abuse, NIH = National Institutes of Health, NIHR = National Institutes of Health Research, SSA = Society for the Study of Addiction, TCORS = Tobacco Centers of Regulatory Science, UKCTAS = UK Centre for Tobacco and Alcohol Studies.

Table 14. Associations between harm perceptions and changes in smoking and vaping behaviours (research question 2). All studies are cohort studies

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Brikmanis et al. (2017) (34) US	**Perceived harm of vaping relative to smoking**. On a scale, perception that smoking is much more unhealthy (1) to vaping is much more unhealthy (5). **Young adult monthly smokers** (n=348) 9 month follow-up	**VAPING FREQUENCY. Perceiving less harm from vaping relative to smoking predicted using e-cigarettes more frequently** (d=-0.10, z=-1.97, p=.049). Frequency of vaping was defined as the proportion of days on which e-cigarettes were used, in the past 9 days, adjusting for baseline e-cigarette use in the past 6 months and number of assessment days completed.
Brose et al. (2015) (35) UK	**Perceived harm of vaping relative to smoking**. "Do you think electronic cigarettes are more harmful than regular cigarettes, less harmful, or are they equally harmful to health?" (less harmful ["less harmful than regular cigarettes"] vs. otherwise ["more harmful than regular cigarettes", "equally harmful", "don't know") **Adult past-year smokers who had never tried vaping** (n=1588) 12 month follow-up Adjusted for gender, age group, education, income, smoking status	**VAPING INITIATION (ever). Perceiving vaping to be less harmful than smoking predicted subsequent vaping initiation** (AOR=1.39, 1.08-1.80, p=.011). Vaping initiation was defined as progressing from not vaping 'at all' to vaping at least 'less than monthly'.

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Chaffee & Cheng (2018) (36) US	**Reduction in perceived harm from vaping (absolute harm)**. "How much do you think people harm themselves when they use e-cigarettes?" ("No harm, "A little harm", "Some harm", A lot of harm"). Decrease in perceived harm was defined choosing a lower level of harm at follow-up than at baseline (e.g., "some" to "a little").* **Youth never smokers or vapers** (n=8005) 12 month follow-up Adjusted for gender, age group, race/ethnicity, region, sensation seeking, home tobacco use, alcohol ever use, tobacco advertisement receptivity, parental education attainment, initiation of other tobacco products. *Note that the way the exposure was coded (change between baseline and follow-up) make it difficult to determine the temporality of the exposure vs. outcome.	**SMOKING AND VAPING INITIATION (ever). Reductions in the perceived harm of e-cigarettes was positively associated with initiating vaping, but not initiating smoking: vaping initiation** (AOR=2.90, 2.12-3.97), smoking initiation (AOR=1.11, 0.69-1.77). Initiation was defined as progressing from never to ever use.
Chen et al. (2018) (37) UK	**Perceived harm of vaping relative to smoking (less harmful than smoking)**. "Is using e-cigarettes less harmful, about the same, or more harmful than smoking cigarettes?" ("Less harmful" vs. "About the same"/"More harmful." **Young adult past month vapers** (n=1421) 12 month follow-up	**VAPING NON-TOBACCO/MENTHOL FLAVOURED E-CIGARETTES. Perceiving that vaping is less harmful than smoking was positively associated with vaping non-tobacco/menthol flavoured e-cigarette vs. tobacco/menthol flavoured e-cigarettes** (AOR=1.59, 1.15-2.19, p=.005). Adjusted for age, sex, race, income, education, sexual orientation, mental health, past-month marijuana use, past-month vaping, smoking status

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Chen-Sankey et al. (2019) (38) US	**Perceived harm from vaping (absolute harm)**. "How much do you think people harm themselves when they use e-cigarettes?" (no harm, little harm, some harm, a lot of harm) **Youth** (n=6983) 12 month follow-up Adjusted for age, gender, race/ethnicity, parental education, sensation seeking, internalized problems, externalized problems.	**VAPING INITIATION (ever and past 30-day). Perceiving vaping causes harm predicted vaping initiation and past 30-day vaping**. 1. Compared with those who perceived that vaping causes a lot of harm, those who perceived that vaping causes some harm (AOR=1.31, 1.01-1.71), little harm (AOR=2.59, 1.97-3.39), or no harm (AOR=2.75, 2.02-3.77) had greater odds of subsequently initiating vaping. 2. Compared with those who perceived that vaping causes a lot of harm, those who perceived that vaping causes little harm (AOR=2.98, 1.77-5.03) or no harm (AOR=4.43, 2.50-7.86) had greater odds of subsequently being past 30-day vapers. There was no difference in the odds of being a past 30-day smoker among those who perceived that vaping causes a lot of harm vs. some harm (AOR=1.30, 0.81-2.09). Vaping initiation was defined as progressing from never vaping to ever vaping, or never to past 30-day vaping. Note that these associations were not presented in the article but were provided by the study's authors.

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Choi & Forster (2014) (39) US	**Perceived harm and addictiveness of vaping relative to smoking, perception that vaping can help people quit smoking**. Agreement with the statements (strongly agree/agree vs. not sure/disagree/strongly disagree for each): Using e-cigarettes is less harmful to health of the user than smoking cigarettes E-cigarettes are less addictive than cigarettes Using e-cigarettes can help people quit smoking **Young adult never vapers** (n=1379) 12 month follow-up Adjusted for age, gender, education, smoking status.	**VAPING INITIATION (ever). Perceiving that vaping is less harmful than smoking, can help people quit smoking predicted subsequently initiating vaping**. Perceiving that vaping is less addictive than smoking did not predict initiating vaping. Perceiving that vaping is less harmful than smoking (AOR=2.34, 1.49-3.69, p<.05), perceiving that vaping can help people quit smoking (AOR=1.98, 1.29-3.04, p<.05), perceiving that e-cigarettes are less addictive than cigarettes (AOR=1.16, 0.73-1.85, p>.05). Associations did not vary by gender or smoking status. Vaping initiation was defined as progressing from never to ever vaping.

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Cooper et al. (2018) (40) US	**Perceived harm and addictiveness of vaping**. 1. Perceived harm: "How harmful are ENDS products to health?" (1-4, from "not at all harmful" to "extremely harmful") 2. Perceived addictiveness: "How addictive are ENDS products?" ("not at all addictive", "somewhat addictive", "very addictive" **Young adult never vapers** (n=2565) 6-24 month follow-up Adjusted for age, sex, race/ethnicity, current smoking, other tobacco product use, use of other substances, type of college attended	**VAPING INITIATION (ever). Lower perceived addictiveness and harms from vaping predicted vaping initiation among non-smokers**. 1. Perceived addictiveness: Lower perceived addictiveness was associated with increased odds of e-cigarette initiation (OR=1.26, 1.08-1.46, p=.003). There was an interaction between current smoking and perceived addictiveness such that lower perceived addictiveness was associated with greater odds of initiation among non-smokers (OR=1.34, p<.001), but not among current smokers (OR=0.90, p=.553). 2. Perceived harm: Lower perceived harm from e-cigarettes was not associated with e-cigarette initiation overall (OR=1.06, 0.95-1.18); however, there was an interaction between current smoking and perceived harm such that lower perceived harm of vaping was associated with greater odds of initiation among non-smokers (OR=1.13, p=.047), but not among current smokers (OR=0.77, p=.062). Vaping initiation was defined as progressing from never to ever vaping.

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Elton-Marshall et al. (2020) (41) US	**Perceived harm of vaping relative to smoking**. Perception that vaping is more harmful (vs. less/the same/don't know), or less harmful (vs. more/the same/don't know) relative to smoking. **Adults** (n=20628 in analyses reported here) 12 month follow-up Adjusted for gender, age, race/ethnicity, sexual orientation, educational attainment, income, smoking status	**VAPING INITIATION/CONTINUED USE. Perceiving vaping as less harmful than smoking predicted vaping initiation/continued use at follow-up**, among baseline users and non-users (OR = 1.97, 1.74-2.22). Vaping was defined as either transitioning from a non-user to a user, or remaining a user. Use was defined as vaping "every day" or "some days".
Harlow et al. (2019) (42) US	**Perceived harm of vaping relative to smoking (more harmful than smoking)** **Adult smokers who did not currently vape** (n=6592) 12 month follow-up Adjusted for age, sex, region	**VAPING INITIATION, AND SMOKING TRANSITIONS. Perceiving that vaping is more harmful than smoking was associated with reduced odds of initiating vaping and becoming a dual smoker/vaper, but not with initiating exclusive vaping or quitting smoking**. 1. Currently vaping every day, some days, or experimentally at follow-up from not vaping every day, some days, or experimentally at baseline (AOR=0.38, 0.24-0.61) 2. Four mutually exclusive categories for transitions: 1) did not begin vaping and continued smoking [no transition] (reference category), 2) initiated vaping and continued smoking [dual smoker/vaper] (AOR=0.41, 0.25-0.65), 3) initiated vaping and quit smoking [exclusive vapers] AOR=0.13, 0.01-1.91), 4) did not initiate vaping and quit smoking [former smokers] (AOR=0.99, 0.64-1.54).

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Hendricks et al. (2018) (43) US	**Perceived harm and addictiveness of vaping relative to smoking**. Scores on perceived health risks of vaping subtract scores on perceived health risks from smoking, and scores on perceived addiction of vaping subtract scores on perceived addiction of smoking. Risks were assessed by asking respondents how likely 1) health risks, and 2) perceived craving/addiction, are from vaping and using tobacco cigarettes (responses from 0 [“completely unlikely”] to 9 [“completely likely”]). **Adult current smokers** (n=978) 12 month follow-up Adjusted for gender, age, race, educational attainment, marital status, Charlson Comorbidity Index, study condition	**VAPING FREQUENCY. Perceiving that vaping poses a risk to health as compared to smoking did not predict change in vaping frequency. Perceiving that vaping satisfies the desire for nicotine as compared to smoking predicted an increase in vaping from 6 to 12 months but not 0 to 6 months.** Perceiving that vaping poses a risk to health as compared to smoking at baseline did not predict subsequent change in number of days used e-cigarettes in the past 30 days at 6 months (path coefficient=- 0.08 p>.05), and at 6 months did not predict subsequent vaping at 12 months (path coefficient=- 0.04 p >.05). Perceiving that e-cigarettes satisfy the desire for nicotine as compared to tobacco cigarettes at 6 months predicted an increase in number of days used e-cigarettes in the past 30 days at 12 months (path coefficient=0.19, p=.04) but not from baseline to 6 months (path coefficient=0.07 p>.05).

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Jayakumar et al. (2020) (44) Canada	**Perceived risk to long-term health of vaping regularly with/without nicotine (absolute harm).** Participants were asked about the risk to long-term health of regularly vaping with and without nicotine (no/slight risk, moderate/great risk) **Young adult never vapers** (n=137) 12 month follow-up	**VAPING INITIATION (ever). Perceiving risk of vaping regularly without nicotine, but not with nicotine, to be moderate/great predicted not initiating vaping:** 1. A lower portion of young people who perceived risk of vaping without nicotine to be moderate or great initiated e-cigarette use (24.1%) compared to those who perceived no or slight risk (44.0%) (unadjusted OR=0.33, 0.13-0.86, p=.023; adjusting for age and sex AOR=0.34, 0.12-0.88, p=.027; adjusting for age, sex, alcohol use, cannabis use, friends vaping, friends smoking, friends using cannabis, seeing someone use e-cigarettes AOR=0.33, 0.11-0.99). 2. Perceiving risk of regularly vaping WITH nicotine as moderate/great (40.2%) vs. no/slight (25.0%) was no different among youth who initiated e-cigarette use (unadjusted OR=2.01, 0.39-10.39, p=.403; adjusting for age and sex AOR=2.13, 0.41-11.13, p=.371). Vaping initiation was defined as progressing from never to ever vaping.
Malt et al. (2020) (45) US	**Perceived harm of vaping relative to smoking.** "Is using e-cigarettes less harmful, about the same, or more harmful than smoking cigarettes?" ("less harmful" vs. otherwise ["about the same", "more harmful") **Adults** (n=21693) – only data from among former smokers who currently vape are reported here, but sample size for this subgroup is not reported 12 month follow-up	**SMOKING RELAPSE. Perceiving that vaping is equally or more harmful than smoking (vs. less) was associated with higher rates of relapse to smoking.** Former smokers who vaped had higher rates of relapsing to smoking if they perceived vaping as being equal to (29%) or more harmful (37%) relative to cigarettes, compared to those who perceived vaping as less harmful (19%) (p<.001). Relapse was defined as transitioning from being a former smoker to a current smoker.

McKelvey et al. (2021) (46) US	**Perceived short- and long-term risks of vaping (absolute harm).** 1. Short-term risks: “Whether or not you have used any of the products, imagine that you just began using one of the products below [e-cigarettes]. You use it about 2-3 times a day, every day. Sometimes you use it alone and sometimes you use it with friends”. Participants selected the perceived percent chance (from 0% to 100%) of: (a) becoming addicted, (b) being able to quit whenever they want, (c) still using the product in 5 years, (d) feeling jittery/nervous, (e) having a bad cough, (f) suffering from more colds, (g) having trouble catching their breath, (h) developing mouth sores, (i) having worse performance in sports, (j) friends being upset with them, (k) feeling high or buzzed, (l) getting in trouble, and (m) having bad breath. 2. Long-term risks: “Imagine now that you continue to use one of the products below [e-cigarettes] 2-3 times a day, every day for the rest of your life”. Participants selected the perceived percent chance (from 0% to 100%) of: (a) developing oral cancer, (b) getting wrinkles, (c) having a heart attack, (d) developing lung cancer, (e) developing another tobacco-related illness, and (f) death from a tobacco-related illness.	**SMOKING AND VAPING INITIATION/ESCALATION. Perceived risks from vaping were negatively associated with vaping, but not smoking, initiation and escalation:** vaping initiation (short-term: tau-b=-0.12, p<.05; long-term: tau-b=-0.10, p<.05; initiation defined as progressing from never to ever use), vaping escalation (short-term: tau-b=-0.09, p<.05; long-term: tau-b=-0.09, p<.05; escalation defined as an increase in number of times product was used, among ever users), smoking initiation and escalation (all p>.05).

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
	Young adults (n=772) Five year follow-up total, but outcomes assessed from 24-36 months after the exposure. Adjusted for age.	
Moustafa et al. (2021) (33) US	**Perceived harm and addictiveness of vaping relative to smoking.** Mean of two items (from 0 [strongly disagree] to 3 [strongly agree]): E-cigarettes might be less harmful for people to be around than cigarettes E-cigarettes might be less addictive than cigarettes. **Youth** (n=1539) 6 month follow-up Adjusted for past 30-day nicotine vaping, past 30-day marijuana vaping, sex, race, ethnicity, peer vaping acceptance, sensation seeking, past 6-month smoking.	**PAST 30-DAY VAPING. Perceiving less harm and addictiveness from vaping relative to smoking predicted an increase in past 30-day nicotine vaping** (unadjusted OR=1.61, 1.12-2.31; AOR=1.61, 1.08-2.41). Past 30-day vaping was assessed as being a past 30-day vaper at follow-up (yes/no) vs. baseline (yes/no).

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Nicksic et al. (2019) (47) US	**Perceived harm of vaping relative to smoking.** "Is using e-cigarettes less harmful, about the same, or more harmful than smoking cigarettes" ("about the same"/"more harmful" vs. "less harmful") Other info: Measured at W1 **Youth never smokers or vapers** (n=5156) 12 month follow-up Adjusted for susceptibility to vaping, susceptibility to smoking, academic performance, sensation seeking, family tobacco use, secondhand smoke exposure, exposure to tobacco advertising, sex, age, race/ethnicity, parent education.	**SMOKING AND VAPING INITIATION (ever and past 30-day). Perceiving vaping as less harmful than smoking was positively associated with initiating ever vaping but not past 30-day vaping or initiating smoking or past 30-day smoking:** initiating ever vaping (AOR=1.57, 95% CI=1.17-2.11), past 30-day vaping (AOR=1.36, 0.79-2.32), ever smoking (AOR=0.96, 0.63-1.47), past 30-day smoking (AOR=0.72, 0.38-1.36). Initiation was defined as progressing from never to ever vaping, or never to past 30-day vaping.

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Parker et al. (2018) (48) US	**Perceived harm of vaping relative to smoking, and perceived harm of vaping (absolute harm).** 1. Harm perception of vaping relative to smoking: "Is using e-cigarettes less harmful, about the same, or more harmful than smoking cigarettes?" ("less harmful" "about the same," "more harmful"). 2. Harm perception of vaping: "How much do you think people harm themselves when they use e-cigarettes?" ("a lot of harm" vs. "no harm"/"a little harm"/"some harm"). **Youth never smokers or vapers** (n=10081) 12 month follow-up Adjusted for age, sex, race, region, parental education, ever alcohol use, ever tobacco use.	**VAPING INITIATION (ever). Perceiving that vaping is less harmful than smoking, and perceiving that vaping poses no/a little harm, or some harm, (vs. a lot of harm), were positively associated with initiating vaping.** Compared with perceiving that e-cigarettes posed a lot of harm, perceiving that e-cigarettes posed no/a little harm (unadjusted RR=3.0, 2.4-3.8, p<.05; adjusted ARR=2.2, 1.7-2.8, p<.05) and some harm (unadjusted RR=1.6, 1.3-2.1, p<.05; adjusted ARR=1.3, 1.0-1.7, p<.05) at baseline were more likely to have initiated vaping at follow-up. Compared with perceiving that e-cigarettes are more harmful than cigarettes, perceiving e-cigarettes to be less harmful than cigarettes at baseline were approximately twice as likely to initiate vaping at at follow-up (unadjusted RR=2.0, 95% CI=1.4-2.8, p<.05; adjusted ARR=1.6, 1.2-2.2, p<.05). However, e-cigarette initiation was similar among youth who perceived e-cigarettes to be about the same harmfulness as cigarettes vs. more harmful (unadjusted RR=1.2, 0.9-1.8, p>.05; adjusted ARR=1.1, 0.8-1.6, p>.05). Initiation was defined as progressing from never to ever vaping.

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Persoskie et al. (2019) (49) US	**Perceived harm of vaping relative to smoking (less, about the same, more, don't know)** **Adult past 30-day smokers and vapers** (n=2211) 12 month follow-up Adjusted for education, race/ethnicity, age, sex	**SMOKING AND VAPING TRANSITIONS AND FREQUENCY. Dual vapers/smokers who perceive vaping as less harmful than smoking have increased odds of switching to exclusive vaping (i.e., quitting smoking) or remaining dual vapers/smokers, and reduced odds of switching to exclusive smoking compared to dual vapers/smokers with other perceptions.** 1. **Vaping/smoking transitions.** Perceiving vaping as less harmful than smoking (vs. otherwise) increased the odds of becoming an exclusive vaper (i.e., quitting smoking) 7.5% vs. 2.7%; aOR=2.9, 1.7-4.8), increased the odds of remaining a dual user (39.6% vs. 29.9%; aOR=1.5, 1.2-1.8), reduced the odds of becoming an exclusive cigarette smoker (44.8% vs. 59.4%; aOR=0.6, 0.5-0.7) and was not associated with becoming a non-user of both products (8.2% vs. 8.0%; aOR=1.1, 0.7-1.7). 2. **Frequency of smoking.** Perceiving vaping as less harmful than smoking (vs. about the same) was associated with being a past 30-day smoker (84.3% vs. 88.8%, aOR=0.6, 0.4-0.9) and increasing the number of days smoked (beta=1.1, 0.2-1.9), but not with changes in cigarettes per day (beta=1.7, -1.2-4.6) at follow-up. 3. **Frequency of vaping.** Perceiving vaping as less harmful than smoking (vs. about the same) was associated with being a past 30-day vaper (47.0% vs. 33.7%, aOR=1.8, 1.4-2.2) but not changes in the number of days vaped (beta=-2.8, -5.2-0.3) or e-cigarette puffs on last day vaped (beta=4.0, -15.9-23.8) at follow-up.

Strong et al. (2019) (50) US	**Perception of risk of harm (absolute harm) and addiction (addiction risk).** 1) Risk of harm (mean score of 3 items, from 1-3): 1. "How much do you think people harm themselves when they [USE/SMOKE PRODUCT]?" ("No harm or little harm", "Some harm", "A lot of harm" 2. "How long do you think someone has to [USE/SMOKE PRODUCT] before it harms their health?" ("1 year or less than 1 year", "5 or more years", "It will never harm their health") 3. "Is [USING / SMOKING PRODUCT] less harmful, about the same, or more harmful than smoking cigarettes?" ("Less harmful", "About the same", "More harmful"). 2) Risk of addiction: "How likely is someone to become addicted to [PRODUCT]?" (1 – "Low" ["Very" and/or "Somewhat unlikely"], 2 – "Medium ["Neither Likely nor Unlikely"], 3 – "High" ["Somewhat Likely" and/or "Very Likely"]) Additional categories were created for youth who reported "Don't know" or who had not heard of vaping, for both the perceived risk and perceived addiction items. **Youth** (n=10081; however, pairwise deletion was used for analyses and so n=9142 when assessing perceived risk and n=9150 when	**VAPING INITIATION (ever). Perceived risk of harm and addiction from vaping were inversely associated with initiating vaping** (risk of harm: F=24.7, p<.001; risk of addiction: F=18.0, p<.001). 1. The probability of initiating vaping was 14% (95% CI: 12-15%) among youth in the "low" category of perceived harmfulness, 8% (7-10%) among youth in the "medium" category, 6% (5-7%) among youth in the "high" category, 5% (4-7%) among youth who did not know if the product was harmful, and 3% (2-4%) among youth who had never heard of e-cigarettes. 2. The probability of initiating vaping was highest among youth in the "low" category of perceived harmfulness, followed by youth in the "medium" category, youth in the "high" category, youth who did not know if the product was harmful, and lowest among youth who had never heard of e-cigarettes. Precise percentages for addiction are not stated. Initiation was defined as progressing from never to ever vaping.

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
	assessing perceived addictiveness) 12 month follow-up Adjusted for age, race/ethnicity, sex	
Vallone et al. (2020) (51) US	**Perceived harm of vaping relative to smoking.** "Compared to regular cigarettes, do you think that e-cigarettes, e-hookah, vape pens, hookah pens, and vape pipes (including JUUL) are" (less harmful, about the same, more harmful, don't know/refused) **Young adults** (n=12114 * the n included in the e-cigarette naïve sample at baseline is not provided and likely differs) 12 month follow-up	**VAPING INITIATION (ever and past 30-day JUUL use). Perceiving vaping is less harmful than smoking predicted past 30-day, but not ever, JUUL initiation.** 1. Past 30-day initiation: Compared with those who perceived vaping to be less harmful than smoking, those who perceived vaping to be about the same (AOR=0.42, 0.23-0.74, p<.01) or more harmful (AOR=0.32, 0.13-0.78, p<.05) than smoking were less likely to initiate past 30-day JUUL use. 'Don't know' responses did not differ from 'less harmful' responses in terms of initiation of past 30-day JUUL use (AOR=0.23, 0.05-1.20, p>.05). 2. Ever initiation: Compared with those who perceived vaping to be less harmful than smoking, those who perceived vaping to be about the same (AOR=0.78, 0.51-1.17, p>.05) or more harmful (AOR=0.90, 0.55-1.48, p>.05) than smoking, and those who did not know the harms (AOR=0.56 (0.26-1.21), p>.05), were no more or less likely to initiate ever JUUL use. Initiation was defined as progressing from never to ever JUUL use, or never to past 30-day JUUL use.

Authors and country	Methods: exposure, sample description, follow-up, covariates adjusted for (if applicable)	Outcomes: measurement and association with exposure
Yong et al. (2014) (52) UK and Australia	**Perceived harm of vaping relative to smoking.** Perception that vaping is more harmful, less harmful, or equally harmful as smoking to one's health. **Adult current or former smokers who had never tried vaping** (n=1590) 24-36 month follow-up Adjusted for country, age group, gender, education, income, minority ethnic/racial group, smoking status, interested in quitting, survey mode, wave of recruitment	**VAPING INITIATION (ever). Perceiving vaping to be equally/more harmful than smoking predicted subsequently not initiating vaping** (AOR=0.41, 0.20-0.83, p<.05). Not knowing whether vaping is less, equally, or more harmful than smoking was not associated vaping initiation (AOR=0.58, 0.32-1.04, p>.05). Initiation was defined as progressing from never to ever vaping.
Zheng et al. (2021) (53) US	**Perception of how much people could harm themselves by vaping (absolute harm)** (no harm, little harm, some harm, a lot of harm) **Youth** (n=6208) 24 months – vaping behaviour assessed at 0 and 24 months, vaping harm perceptions assessed at 12 months Adjusted for vaping status, gender, age, race/ethnicity, parent education, number of best friends who vape, self-perceived physical health status	**PAST 30-DAY VAPING. Perceptions of vaping harms were negatively associated with past 30-day vaping** (AOR=0.57, 95% CI=0.48-0.68, p<.001). Past 30-day vaping was defined as any vaping in the past 30 days, adjusting for past 30-day vaping at baseline.

15.6 Conclusions

Harm perceptions in England

Among 11 to 18 year olds (ASH-Y), just under half (44.7%) accurately perceived that vaping was less harmful than smoking; around a third (32.4%) inaccurately thought that the harms from vaping and smoking were about the same; 3.6% inaccurately thought that vaping was more harmful than smoking; and 19.3% said they did not know.

The proportion of 11 to 18 year olds who accurately thought that vaping was less harmful than smoking declined from 66.7% in 2015 to 43.3% in 2020, and then increased slightly in 2021 to 44.7%. The proportion not knowing has increased from 9.9% in 2015 to 19.3% in 2021.

Among 11 to 18 year olds, inaccurate perceptions that vaping is more or equally as harmful as smoking were similar between those who currently vaped and those who never vaped. Only half of current smokers aged 11 to 18 years accurately perceived vaping as less harmful than smoking.

Among 16 to 19 year olds (using ITC Youth data), slightly different patterns were observed in 2021, with most (62.9%) accurately perceiving vaping is less harmful than smoking, 16.8% inaccurately perceiving vaping and smoking to be equally harmful, 10.0% inaccurately perceiving vaping to be more harmful than smoking and 10.0% reporting that they didn’t know.

In relation to absolute harms, young people (16 to 19 year olds) rated smoking daily higher on the scale of harm than smoking on some days (88.0% compared with 65.2% rating it ’very’ or ‘extremely’ harmful); however, there was less difference between young people’s perceptions of vaping daily and vaping on some days (31.9% and 22.6% respectively). Slightly greater proportions of young people perceived some day or daily vaping as not at all harmful (6.2% and 2.8% respectively) than they did for smoking (both 0.6%), although proportions for perceiving vaping or smoking as not at all harmful were very small. A greater proportion of young people did not know the harms of vaping (about 11.5%) than did not know the harms of smoking (less than 1%).

Half of 16 to 19 year olds perceived vaping to be ‘slightly’ or ‘somewhat’ addictive (50.7%), one-third perceived vaping to be ‘very’ or ‘extremely’ addictive (31.7%), and few (6.3%) perceived e-cigarettes to be ‘not at all’ addictive with 11.1% saying they did not know.

Over half of 16 to 19 year olds perceived that vaping makes quitting smoking permanently ‘a bit’ or ‘a lot easier’ (60.0%); many (14.2%) thought it had ‘no effect’, just under one-tenth (9.6%) perceived that vaping made quitting ‘a bit’ or ‘a lot harder’, with 15.9% saying that they did not know.

Overall, just over half of 16 to 19 year olds reported noticing any education campaign or public health message about vaping in the past 12 months (53.0%).

Among adult smokers in 2021 STS data, just over a third (34.1%) accurately perceived that vaping was less harmful than smoking, around a third (32.1%) inaccurately thought that the harms from vaping and smoking were about the same, 11.9% inaccurately thought that vaping was more harmful than smoking, and 22.0% said they did not know.

The proportion of adult smokers who inaccurately perceived that vaping was more harmful or equally harmful than smoking has declined since 2020 by 2.9 and 5.6 percentage points, respectively. The proportion of smokers who accurately perceived that vaping is less harmful than smoking increased by 5.0 percentage points since 2020 (the first time an increase in this measure had been observed since 2014). However, there seems to be growing confusion regarding the relative harms of vaping compared with smoking: STS found that the proportion of adult smokers who said that they did not know whether smoking or vaping was more harmful has more than doubled from 9.5% in 2019 to 22.0% in 2021.

In the ASH-A survey, overall, few (13.9%) current adult smokers and vapers accurately believed that none or a small amount of the risks of smoking were due to nicotine, with 23.9% reporting under half the risk, 17.3% around half the risk, 26.9% much more than half or nearly all the risk, and 18.1% did not know.

There was a notable gradual increase in correct nicotine harm perceptions among adults depending on participants' experience with vaping: 10.8% of current smokers, 15.6% of smokers and vapers, and 20.3% of exclusive vapers accurately reported that none or a very small amount of the health risks from smoking come from nicotine in tobacco cigarettes.

Systematic review of vaping harm perceptions: examining interventions to change them, and longitudinal associations with vaping and smoking behaviours

We have included a systematic review of vaping harm perceptions examining interventions to change them, and longitudinal associations with vaping and smoking behaviours.

Interventions to change perceptions

We identified 32 articles (from 29 studies) addressing our first research question: what interventions have been effective in changing harm perceptions?

Studies involved either adults or young people, and addressed relative perceptions of the harms of vaping (compared with smoking), absolute perceptions of the harms of vaping or

addictiveness (such as the perception that e-cigarettes contain harmful chemicals, cause heart disease or cancer, or that vaping is addictive), or perceptions of the harms of nicotine (including perceived addictiveness of nicotine).

Of the 32 articles, 13 (from 10 studies) assessed interventions involving written information about vaping. Of these, 6 focused on relative harms, 3 focused on nicotine (varied messages), and 5 focused on absolute harm and addictiveness messages (for example, that e-cigarettes contain harmful chemicals, and that nicotine is an addictive chemical). Several studies focussed on more than one type of message. This included the following.

1. Of the 6 articles (from 5 studies) providing written information about the reduced harms of vaping relative to smoking, 5 found statistically significant associations with an increase in accurately perceiving that vaping is less harmful than smoking, but also in one study a decrease in absolute harm perceptions, but in another study an increase in absolute harm perceptions for some, but not all, smoker subgroups.

2. Of the 3 articles (from 2 studies) providing written information about nicotine, all 3 found statistically significant associations with changing perceptions of nicotine according to the messages given (for example, not the main cause of smoking-related health problems, addiction).

3. Of the 5 articles providing written information about the absolute harms of vaping (sometimes including addictiveness), 3 found statistically significant associations with increased absolute and/or relative vaping harm perceptions (that is, perceiving vaping as harmful to health, risk of developing specific diseases, and vaping as equally/more harmful than smoking).

Four studies assessed educational workshops/videos designed to deter vaping through providing information about the absolute harms of vaping (for example, risk of heart disease, cancers, respiratory diseases) and addictiveness. Of these 4, 3 found statistically significant associations with increased absolute (including addictiveness) and/or relative vaping harm perceptions.

Five studies assessed mass media campaigns or advertisements, of which 2 focused on relative harms, 2 focused on both absolute and relative harms, and one focused on a youth vaping prevention campaign. Of these 5, 3 found statistically significant associations with vaping harm perceptions. The youth vaping prevention campaign increased perceptions of absolute vaping harms, self-reported exposure to information refuting incorrect claims about vaping reduced perceptions of relative and absolute harms, and advertisements promoting vaping as a better/healthier alternative to smoking increased perceptions of vaping as healthy.

Three studies assessed warning labels/packaging, of which one focused on both absolute and relative harms, one focused on absolute harm and nicotine addiction, and one focused

on relative harm and nicotine addiction. Findings were mixed overall. Regarding comparative warning labels, those focusing on the reduced harm of vaping relative to smoking decreased absolute harm perceptions of vaping in one of 2 studies, those focusing on the equal harm of vaping relative to smoking increased absolute harm perceptions of vaping in the one study assessing this association, but no studies assessed the impact of comparative warning labels on relative harm perceptions. Warning labels focusing on the absolute harm of vaping increased absolute vaping harm perceptions in one of 2 studies. Warning labels focusing on the addictiveness of nicotine increased absolute vaping harm perceptions including addictiveness in both studies assessing this association, but did not change relative harm perceptions including relative addictiveness in the one study assessing this outcome.

Three studies assessed video games aimed to prevent youth vaping, all 3 of which found statistically significant associations with increased perceptions of absolute harm, and one of which found a statistically significant association with perceived addictiveness of vaping (from the 2 studies assessing this outcome).

Four studies assessed whether vaping harm perceptions changed after EVALI outbreak, all 4 of which found statistically significant associations with increases in the absolute or relative harm perceptions (sometimes including addictiveness) of vaping.

In summary, our review found that interventions communicating information about the reduced harms of vaping relative to smoking generally increased people’s perceptions that vaping is less harmful than smoking. Most of this evidence came from studies of adults.

We also found that interventions communicating information about the absolute harms of vaping (sometimes including the risks of addiction and developing specific diseases/health ailments) generally increased the perception that vaping is harmful to health, can lead to diseases/health ailments, and is equally or more harmful relative to smoking. Most of these interventions were aimed at youth or young adults specifically to deter vaping through providing information about vaping risks.

EVALI also increased harm perceptions of vaping including inaccurate perceptions relative to smoking.

Warning labels highlighting that vaping is harmful and addictive generally increased perceptions that vaping is harmful to health and is addictive.

Vaping harm perceptions predicting changes in behaviour

We identified 21 studies addressing our second research question: to what extent are vaping harm perceptions predictive of any changes in vaping and smoking behaviours?

Among youth and young adults, 14 studies assessed associations between vaping harm perceptions and changes in vaping behaviours, of which all 14 found statistically significant associations. Specifically, perceiving vaping as less harmful than smoking (sometimes including perceived addictiveness) and lower perceptions of vaping harms (absolute harm) predicted subsequent increases in vaping (for example, trying vaping among baseline never vapers, increase in number of days vaped and past 30-day vaping among predominantly non-smokers and non-vapers). Lower perceived harm of addiction from vaping and the perception that vaping can help people quit smoking predicted subsequently trying vaping among baseline never vapers.

Also among youth and young adults, 3 studies (2 of which also assessed vaping behaviours) assessed associations between vaping harm perceptions and changes in smoking behaviours, of which none found statistically significant associations. Specifically, perceived relative harm of vaping compared with smoking and perceived absolute harm of vaping (sometimes including perceived risk of addiction) were not statistically significantly associated with subsequently trying smoking among baseline never smokers, and perceived absolute harm was also not associated with escalating smoking among ever smokers.

No studies among youth or young adults assessed whether vaping harm perceptions predicted switching away from smoking to vaping.

Among adults, 6 studies assessed associations between vaping harm perceptions and changes in vaping behaviours, of which 5 found statistically significant associations. Specifically, perceiving vaping as less harmful than smoking predicted subsequent increases in vaping (for example, trying vaping among baseline never vapers who smoke/formerly smoked, continued vaping among current smokers).

Also among adults, 3 studies assessed associations between vaping harm perceptions and changes in smoking behaviours, 2 of which found statistically significant associations. Specifically, perceiving vaping as equally or more harmful than smoking was statistically significantly associated with subsequent relapse to smoking among former smokers in one study, while perceiving vaping as less harmful than smoking predicted quitting smoking in the other study.

In summary, vaping harm perceptions consistently predicted subsequent changes in vaping behaviours among youth, young adults, and adults, consistent with normal expectations for approaching lower harm and avoiding greater harm. Perceiving vaping as less harmful than smoking predicted subsequent increases in vaping (including ever starting vaping) among youth and young adults, but also among adults and adult smokers. Conversely, perceiving vaping as harmful was associated with not initiating vaping among youth and young adults.

Substantially fewer studies assessed whether people's vaping harm perceptions predicted subsequent changes in their smoking behaviours. However, the limited evidence suggests that perceiving vaping as equally or more harmful than smoking predicted subsequent relapse to smoking among adult former smokers. Also, perceiving vaping as less harmful than smoking predicted quitting smoking. But among youth and young adults, relative and absolute harm perceptions (sometimes including perceived risk of addiction) were not associated with starting smoking. Absolute harm perceptions were not associated with smoking more.

In general, the findings were broadly consistent with people's normal expectations for approaching what they perceive to be lower harm and avoiding what they perceive to be greater harm.

Taken together, findings suggest that messages about the harms of vaping influence vaping perceptions. This in turn impacts vaping and smoking behaviours.

Providing information aimed to deter vaping among youth (for example, highlighting the harms of vaping) can increase perceptions of the harm of vaping to health, which in turn can deter trying vaping among youth. Conversely, providing information aimed to increase accurate relative perceptions of vaping compared to smoking can increase accurate relative perceptions of vaping compared with smoking, which in turn could lead adult smokers to try vaping, reduce risk of relapse to smoking among adult former smokers who vape, but it could also could lead to youth trying vaping.

The effects of vaping harm perceptions on longer-term vaping, smoking, and vaping as a substitute for smoking, remain unclear. More high-quality studies and those that assess whether changes in vaping harm perceptions and vaping and smoking behaviours are maintained over time are required.

Risk of bias was high for all included studies for both our research questions. All randomised and non-randomised studies addressing our first research questions employed a pre-post design with no longitudinal follow-up. For our second research question, the vast majority of studies had a follow-up length of 12 months or less. More high-quality studies and those that assess whether changes in vaping harm perceptions and changes in vaping and smoking behaviours are maintained over time (particularly into adulthood) are required.

15.7 Implications

Given a substantial proportion of young people, and adult smokers and vapers in England still hold inaccurate perceptions of the relative harms of vaping compared with smoking (that vaping is equally or more harmful than smoking), these misperceptions need to be addressed.

Providing accurate information about the relative harms of vaping, and risks of using nicotine, could help to correct misperceptions of vaping and nicotine, respectively, particularly among adults.

Interventions on absolute harms of vaping need to be carefully designed so as not to misinform young people (particularly smokers) about the relative harms of smoking and vaping.

Warning labels highlighting that vaping is harmful and addictive generally increased perceptions that vaping is harmful to health and is addictive. No studies assessed the effects of warning labels highlighting the relative harms of smoking and vaping, on relative harm perceptions. So, these studies are needed.

Vaping harm perceptions consistently predicted subsequent changes in vaping behaviours among youth, young adults, and adults, consistent with normal expectations for approaching lower harm and avoiding greater harm. Perceiving vaping as less harmful than smoking predicted subsequent increases in vaping (including initiation of ever vaping) among youth and young adults, but also among adults and adult smokers. Conversely, perceiving vaping as harmful was associated with not initiating vaping among youth and young adults. Substantially fewer studies assessed whether vaping harm perceptions predicted subsequent changes in smoking behaviours; however, the evidence suggests that perceiving vaping as equally or more harmful than smoking predicted subsequent relapse to smoking among adult former smokers, while, among youth and young adults, relative and absolute harm perceptions were not associated with smoking initiation or escalation. No studies among young people or young adults assessed whether vaping harm perceptions predicted subsequent switching from smoking to vaping, or the other way around. So, more high-quality studies and studies addressing substituting smoking with vaping in young people, young adults and adults are needed.

More longitudinal randomised studies assessing interventions to change vaping harm perceptions are needed. There is also a need for studies that assess whether changes in vaping harm perceptions (in response to interventions) and vaping and smoking behaviours (associated with harm perceptions) are maintained over time (particularly into adulthood). Communications about absolute and relative harms of vaping and smoking are likely to reach both youth and adults. From an ethical standpoint, the main aim of these communications must be to ensure that the messages give accurate information about absolute harms of vaping, and the relative harms of vaping as compared to smoking, so as to address the prevalent misperceptions. Messages will need to be carefully developed and nuanced to avoid unintended effects (for example, ‘less harmful’ translating to a perception of ‘safe’) and should be tested on target audiences first. Finally, continued surveillance of perceptions in young people and adults is needed.

15.8 Appendix 1

Search strategy

The search strategy involved 3 key concepts (combined with AND):

1. Risk or harm (risk* OR harm* OR health* OR safe* OR danger* OR hazard* OR toxic* OR addict* OR damage*).

2. Perception (perception* OR perceive* OR belief* OR believe* OR attitude* OR opinion* OR approv* OR disapprov* OR accept* OR unaccept* OR aware*).

3. Vaping (Electronic Cigarettes OR e-cig* OR electronic cig* OR (ENDS AND Nicotine) OR electronic nicotine delivery system* OR (Nicotine AND (Vaping* OR Vape* OR Vapor* OR Vapouris*))).

All searches were limited to:

- January 2007 to July 2021
- humans

Search terms

Medline (via PubMed)

(risk* OR harm* OR health* OR safe* OR danger* OR hazard* OR toxic* OR addict* OR damage*) AND (perception* OR perceive* OR belief* OR believe* OR attitude* OR opinion* OR approv* OR disapprov* OR accept* OR unaccept* OR aware*) AND (Electronic Cigarettes OR e-cig* OR electronic ciga* OR (ENDS AND Nicotine) OR electronic nicotine delivery system* OR (Nicotine AND (Vaping* OR Vape* OR Vapor* OR Vapouris*)))

All fields selected, which captures keywords in all fields.

CIHAHL (via EBSCO)

((risk* OR harm* OR health* OR safe* OR danger* OR hazard* OR toxic* OR addict* OR damage*)) AND ((perception* OR perceive* OR belief* OR believe* OR attitude* OR opinion* OR approv* OR disapprov* OR accept* OR unaccept* OR aware*)) AND ((Electronic Cigarettes OR e-cig* OR electronic ciga* OR (ENDS AND Nicotine) OR electronic nicotine delivery system* OR (Nicotine AND (Vaping* OR Vape* OR Vapor* OR Vapouris*))))

No field selected, to capture keywords in the title, abstract, and subject headings.

PsycInfo AND EMBASE (via Ovid)

((risk* or harm* or health* or safe* or danger* or hazard* or toxic* or addict* or damage*) and (perception* or perceive* or belief* or believe* or attitude* or opinion* or approv* or disapprov* or accept* or unaccept* or aware*) and (Electronic Cigarettes or e-cig* or electronic ciga* or (ENDS and Nicotine) or electronic nicotine delivery system* or (Nicotine and (Vaping* or Vape* or Vapor* or Vapouris*)))).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

Multipurpose field selected (.mp), which captures keywords in the title, abstract, subject heading, name of substance, and registry word fields.

SCOPUS

(risk* OR harm* OR health* OR safe* OR danger* OR hazard* OR toxic* OR addict* OR damage*) AND (perception* OR perceive* OR belief* OR believe* OR attitude* OR opinion* OR approv* OR disapprov* OR accept* OR unaccept* OR aware*) AND (Electronic Cigarettes OR e-cig* OR electronic ciga* OR (ENDS AND Nicotine) OR electronic nicotine delivery system* OR (Nicotine AND (Vaping* OR Vape* OR Vapor* OR Vapouris*)))

All fields selected, which captures keywords in all fields.

15.9 Appendix 2

Risk of bias – randomised studies (RoB2)

Study	Randomisation process	Deviations from the intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall risk of bias
Booth et al. (2019) (20)	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Calabro et al. (2019) (4)	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
DeHart et al. (2019) (6)	Some concerns	Low risk	Low risk	Low risk	Some concerns	Some concerns
England et al. (2021) (21)	Some concerns	Low risk	Some concerns	Low risk	Some concerns	Some concerns
Keating (2018) (7)	Low risk	Low risk	Some concerns	Low risk	Some concerns	Some concerns
Kimber et al. (2020) (25)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Lee et al. (2018) (26)	Low risk	Low risk	Some concerns	Low risk	Some concerns	Some concerns
Majumdar et al. (2019) (8)	Low risk	Low risk	Some concerns	Low risk	Some concerns	Some concerns
Pepper et al. (2019) (10)	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Popova & Ling (2014) (27)	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Yang & Popova (2020) (11)	Low risk	Low risk	Some concerns	Low risk	Some concerns	Some concerns
Yang et al. (2019) (13)	Low risk	Low risk	Some concerns	Low risk	Some concerns	Some concerns

Study	Randomisation process	Deviations from the intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall risk of bias
Yang et al. (2020) (14)	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Yang et al. (2021) (15)	Low risk	Low risk	Some concerns	Low risk	Some concerns	Some concerns

Risk of bias – non-randomised studies (ROBINS-I)

Study	Confo-unding	Selection of participants	Classification of interventions	Deviations from intervention	Missing data	Measure-ment of outcomes	Bias in selection of the reported result	Overall risk of bias
Yang et al. (2018) (12)	Serious	Low	Low	Low	No information	Moderate	Moderate	Serious on one domain
Baer et al. (2021) (16)	Serious	Low	Low	Low	No information	Moderate	Moderate	Serious on one domain
Bono et al. (2019) (3)	Serious	Low	Low	Low	Low / no information	Moderate	Moderate	Serious on one domain
Carpenter et al. (2021) (5)	Serious	Low	Low	Low	Moderate	Moderate	Moderate	Serious on one domain
Gaiha et al. (2021) (17)	Serious	Low	Low	Low	Moderate	Moderate	Moderate	Serious on one domain
Hieftje et al. (2021) (28)	Serious	Low	Low	Low	Moderate	Moderate	Moderate	Serious on one domain
Little et al. (2016) (18)	Serious	Low	Low	Low	No information	Moderate	Moderate	Serious on one domain
Noar et al. (2019) (9)	Serious	Low	Low	Low	Moderate	Moderate	Moderate	Serious on one domain
Pentz et al. (2019) (29)	Serious	Low	Low	Low	No information	Moderate	Moderate	Serious on one domain
Ratneswaran et al. (2019) (22)	Serious	Low	Low	Low	No information	Moderate	Moderate	Serious on one domain
Sergakis et al. (2019) (19)	Serious	Low	Low	Low	Moderate	Moderate	Moderate	Serious on one domain
Weser et al. (2021) (30)	Serious	Low	Low	Low	No information	Moderate	Moderate	Serious on one domain

Risk of bias – Newcastle Ottawa Scale for cohort studies

Study	Representativeness of exposed cohort	Selection of non exposed cohort	Ascertainment of exposure	Assessment of outcome	Adequacy of follow up	Risk of bias (total number of stars)
Harlow et al. (2019) (42)	1	1	0	0	1	High (3)
Persoskie et al. (2019) (49)	1	1	0	0	0	High (2)
Chaffee & Cheng (2018) (36)	1	1	0	0	1	High (3)
Nicksic et al. (2019) (47)	1	1	0	0	1	High (3)
McKelvey et al. (2021) (46)	0	1	0	0	0	High (1)
Brose et al. (2015) (35)	1	1	0	0	1	High (3)
Yong et al. (2014) (52)	1	1	0	0	0	High (2)
Choi & Forster (2014) (39)	1	1	0	0	1	High (3)
Cooper et al. (2018) (40)	1	1	0	0	1	High (3)
Jayakumar et al. (2020) (44)	1	1	0	0	0	High (2)
Parker et al. (2018) (48)	1	1	0	0	1	High (3)
Strong et al. (2019) (50)	1	1	0	0	0	High (2)
Chen-Sankey et al. (2019) (38)	1	1	0	0	1	High (3)
Vallone et al. (2020) (51)	1	1	0	0	1	High (3)
Elton-Marshall et al. (2020) (41)	1	1	0	0	1	High (3)
Zheng et al. (2021) (53)	1	1	0	0	1	High (3)
Hendricks et al. (2018) (43)	0	1	0	0	1	High (2)
Brikmanis et al. (2017) (34)	0	1	0	0	1	High (2)
Chen et al. (2018) (37)	1	1	0	0	1	High (3)
Malt et al. (2020) (45)	1	1	0	0	0	High (2)
Tan et al. (2015) (23)	0	1	0	0	1	High (2)
Moustafa et al. (2021) (33)	1	1	0	0	1	High (3)

Risk of bias – Newcastle Ottawa Scale for cross-sectional studies

Study	Representativeness of sample	Sample size	Non-respondents	Ascertainment of exposure	Assessment of outcome	Statistical test	Total number of stars
Alber et al. (2021) (31)	1	1	0	1	1	1	5
Morgan et al. (2021) (32)	1	1	1	1	1	1	6
Tattan-Birch et al. (2020) (2)	1	1	0	1	1	1	5
Tattan-Birch et al. (2020) (24)	1	1	0	1	1	1	5

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16 Conclusions

16.1 Preamble

Tobacco smoking is uniquely dangerous, prematurely killing over a half of regular sustained users. Most people who vape have smoked at some time in their life. A prime focus of this report is the potential health risks of vaping both compared to smoking tobacco cigarettes and compared with neither smoking nor vaping. In addition to previous or current smoking history, the overall public health impact of vaping will also depend on the products on the market, how they are regulated, how they are used and by whom, the extent to which they are used as substitutes for smoking, their dependency and the duration of their use and perceptions of their relative and absolute health harms. Our report therefore also gives a snapshot of some of these issues reflecting the extent of knowledge at this time. Nevertheless, as vaping products continue to evolve, and changes are made to their regulatory framework as well as wider contextual issues such as any changes to the regulation of tobacco cigarettes, we recommend that ongoing surveillance of all the above issues is required. Additionally, given the advent of other nicotine products on the market in recent years (for example, heated tobacco products, tobacco-free nicotine pouches), we believe that surveillance and updates on these products are also required to give a complete picture of nicotine and tobacco use.

In this chapter, we have also tried to reflect on changes in England since our first report in 2015, which may also help to understand underlying trends, given the influence of COVID-19 recently on both the availability of data as well as on smoking and vaping behaviours.

Finally, although we addressed all issues posed by our commissioners, we did not cover 2 important issues that we felt were being addressed comprehensively elsewhere or had been covered in our previous reports. First, the relationship between vaping and subsequent smoking, given a new Cochrane review is examining the existing literature about this among those under 30 years old. Secondly, we did not examine the evidence for the effectiveness of vaping for smoking cessation in this report. We have covered this question in our previous report, and the Cochrane collaboration has an ongoing living systematic review entitled ‘Electronic cigarettes for smoking cessation’ which examines the effectiveness of using electronic cigarettes to help people who smoke tobacco achieve long-term smoking abstinence and searches for updates of the evidence monthly.

16.2 Regulatory structures

Generally, in England we have a structure wherein vaping products are available and accessible to adults, while prohibiting access for under 18 year olds. Overall, the regulatory framework has changed little since the translation of the EU Tobacco Products

Directive to UK law through the UK's Tobacco and Related Products Regulations 2016 (TRPR) which came into force from May 2016. Indeed, a recent Post Implementation Review of the UK's TRPR concluded that these regulations had met their original objectives and could not be better achieved through alternative regulatory measures. The review of the 2015 Nicotine Inhaling Products regulations reached a similar conclusion.

In England, a 2-pronged strategy has been adopted for vaping products containing nicotine to have consumer nicotine vaping products and medicinally licensed nicotine vaping products. This is overseen by the Medicines and Healthcare products Regulatory Agency (MHRA). Nicotine-free vaping products are regulated by general consumer legislation which is enforced by local authority (which have a sub-regional footprint) trading standards officers. Trading standards officers enforce consumer legislation in their local areas, which includes advice on consumer law, such as consumer safety and counterfeit goods, investigating complaints and prosecuting traders who break the law. Bodies such as the Consumer Protection Partnership and National Trading Standards facilitate communication across local authority trading standards groups at a national level.

16.3 Consumer vaping products

Some incentives to smokers to switch to vaping are in place, such as the tax structure, as vaping products are not taxed as heavily as cigarette smoking, and advertising restrictions are not as comprehensive as those applied to tobacco cigarette products. Nicotine vaping products which adhere to certain constraints (such as a tank or cartridge capacity of no larger than 2mL, e-liquid refill container capacity of no more than 10mL and a maximum nicotine strength of 20mg/mL) are allowed on the market as consumer products following a notification process to the MHRA. We describe this notification process in chapter 1 (introduction) and chapter 6 (flavours). The MHRA has a public facing database of products that have been notified including a list of withdrawn notifications. An analysis of data recorded in the first year of operation (November 2016 to October 2017) suggested that in most cases products were unlikely to cause serious long-term harm but there were opportunities to minimise potential hazards further.

However, the notification process in place before products can be marketed relies on the oversight of the MHRA and trading standards officers to draw on local intelligence around any products of concern or where there are age of sale violations. We are concerned that trading standards teams have faced increasing financial cuts over recent years and the MHRA appears also to be facing some reduction in staff. Adequate capacity needs to be in place to monitor notifications and to identify and rapidly act on vaping products of concern when they emerge on the market.

In chapter 1 (introduction), we highlight the seizures of illegal disposable vaping products reported by the Society of Chief Officers of Trading Standards and the National Tobacco and Age Restricted Products Groups in Scotland. We are aware from local authority

websites that seizures of illegal vaping products, particularly disposable products that do not adhere to UK regulations, and underage sales are frequently happening in England. The Chartered Trading Standards Institute in England is currently conducting a study of underage access to vaping products, but all this information and intelligence had not, at the time of writing, been pulled together to give an accurate picture of what is currently happening in England. Without an understanding of a national picture and with reduced capacity to act, such illegal products and underage sales may undermine the permissive and supportive structures in England, wherein vaping products are available and accessible to adults who want to transition away from smoking.

In May 2016, the MHRA's Yellow Card scheme (the system for reporting of suspected side effects or adverse drug reactions to any medicines or vaccines, as well as medical device incidents) launched an online reporting form to collect cases of suspected adverse reactions and physical safety concerns associated with nicotine vaping products. In November 2021, the National Institute for Health and Care Excellence recommended that health professionals ask adults who use nicotine-containing vaping products about any side effects or safety concerns that they may experience, and report these via the Yellow Card scheme. Since May 2016, the MHRA has received 257 Yellow Card adverse reaction reports covering 720 adverse reactions; 122 serious reports and 135 non-serious reports have been recorded (14 and 12 respectively between 1 January 2021 and 13 January 2022). Up until January 2022 there were 3 suspected fatalities which were discussed in our 2021 report, with no fatalities reported in the last year (since January 2021). It is not clear how widely known it is that the Yellow Card scheme can be used for reporting suspected adverse reactions to vaping products, and we suggest more widespread promotion of its use. However, as there are just over 3 million people who vape in England, it does not appear that there are widespread safety concerns about vaping products used in England.

The notification system in England would appear to be fit for purpose, with the caveat around the need for resources mentioned above–and it would be helpful if the system of notification enabled regular research updates on the products on the market, for example by providing a more easily searchable database.

16.4 Medicinally licensed vaping products

To complement the consumer regulatory route, there is a process where manufacturers can apply to have a vaping product licensed as a medication. It is perceived that this would increase accessibility of vaping products to people who smoke, enable vaping products to have higher nicotine content than 20mg/mL and also help reassure some smokers and health professionals of the efficacy and relative harms of vaping and smoking. MHRA's initial 2017 guidance on licensing was updated in October 2021 and intended to clarify the requirements, particularly quality standards for dose uniformity and the design of clinical pharmacokinetic studies. However, as we have pointed out since our first report in 2015, it

is disappointing that no such product has come to market and to our knowledge, there have not been any further applications since the MHRA guidance changed.

16.5 Smokefree 2030 and vaping products

The government has set a target to go smokefree by 2030 and the key challenge will be ensuring that smoking prevalence is less than 5% for all groups in society (vaping is not included in smoking prevalence and hence not included in the smokefree target). An independent review into tobacco control by Javed Khan, OBE and a new Tobacco Control Plan under development will set out how the Smokefree 2030 ambition will be achieved. The Smokefree 2030 target is tough and will require an acceleration of the declines in smoking in recent years, particularly in more disadvantaged societal groups.

We summarised the All Party Parliamentary Group on Smoking and Health recommendations for the new Tobacco Control Plan highlighting the recommendations relating to vaping products. These were to make the medicinal licensing for vaping products fit for purpose and to reduce the appeal and availability of vaping products and other nicotine products to children. We identified additional factors that could be important such as bringing nicotine-free vaping products under greater regulatory oversight, and advertising regulations around harm reduction claims and validation which appear to set the barrier too high. In chapter 15 (harm perceptions and communications), we indicated that most consumers are confused about the relative harms of vaping and smoking. So unless this is a key government focus moving forward, the contribution that vaping could make to reducing smoking will not be fully realised.

16.6 International updates

Internationally, there are a wide range of approaches and policies proposed and implemented aimed at regulating nicotine vaping, with little consistency in approach. In the US, the authorisation process for vaping products progressed slowly, and as of 13 May 2022 had issued 21 marketing approvals for vaping products manufactured by 2 tobacco companies and one independent company. In July 2021, Health Canada enacted regulations establishing a maximum nicotine threshold of 20mg/mL for vaping products. In Australia over the last year further changes have been brought in to capture all nicotine vaping products as prescription-only medicines and for the process of issuing nicotine prescriptions. Most notably, the New Zealand government published its Smokefree Aotearoa 2025 Action Plan to reduce daily smoking across all societal groups to 5% by 2025. This included a number of measures aimed at co-regulating the nicotine and tobacco market including a notification process for vaping products.

16.7 Nicotine vaping in England

Vaping among young people

In chapter 3 (vaping among young people), we examined vaping levels among young people in England, and also assessed smoking levels, as the inter-relationship between smoking and vaping enables an examination of how vaping might be affecting smoking in this population.

We describe survey data from 2 sources. The first, among 11 to 18 year olds (ASH-Youth) from 2021 and 2022 (top-line data only) surveys, and the second among slightly older teenagers (16 to 19 year olds; ITC Youth) from February 2021. ASH-Youth data indicated that current smoking prevalence (including occasional and regular smoking) in March 2021 was 4.1% and 6.0% in 2022, compared with 6.7% in 2020 (and 7.1% in 2015); current vaping prevalence ((including occasional and regular vaping) was 4.0% in March 2021 and 8.6% in 2022 compared with 4.8% in 2020 and 2019 (and 1.2% in 2015). The ASH-Youth data suggest that overall current nicotine use (via smoking and/or vaping) was higher in 2022, at 11.1% compared with 6.2% in 2021.

The ITC-Youth data among 16 to 19 year olds identified that current smoking prevalence was 7.9% in February 2021 compared with 8.5% a year earlier and 6.2% in August 2019; current vaping prevalence was 9.1% in February 2021 compared with 9.4% a year earlier, and 7.7% in August 2019. The difference between the 2 surveys in 2021 seemed largely attributable to 19 year olds for whom vaping has been steadily increasing in recent years.

Encouragingly, most young people (around 98%) who had never smoked were also not currently vaping, which indicates there is considerable overlap in the smoking and vaping prevalence figures given above. Disposable models became the most popular type of vaping device in 2022, used by just over half of young people who vaped. This was starkly different to the previous year when disposable vaping products were used by only 7.8% of current vapers.

Overall, these data suggest vaping and smoking among young people appear to have decreased between 2020 and 2021 but then increased in 2022. Hence it important that trends continue to be monitored. Research could also be commissioned into the impact of the changing vaping product market as well as any possible lasting effects of the COVID-19 pandemic on vaping and smoking products. The dramatic increase in use of disposable products should be monitored with improved regulatory oversight and the advertising, packaging and marketing of these products to young people investigated.

In contrast to the adult patterns described in chapter 4 (vaping among adults) and below, we reported that in 2021 smoking and vaping among young people were relatively higher in more socioeconomically advantaged groups. However, in 2022 there was little variation

between social grades in young people. The new Cochrane review will also examine whether the relationship between vaping and smoking differs by socio-economic status and other demographics.

In 2021, among 11 to 18 year olds, fruit flavours were the most popular among current vapers followed by ‘menthol/mint’, then ‘chocolate/dessert/sweet/candy’ flavours, similar to data presented in our 2021 report. Fruit flavours were also the most popular in current vapers, followed by menthol/mint and tobacco among 16 to 19 year olds in the 2021 ITC-Youth survey.

In 2021, vaping nicotine was most common but substantial minorities reported vaping nicotine-free products. As well as stricter enforcement of under-age sales, nicotine-free vaping products need to be brought under stronger regulatory oversight, as there is no notification process for such products meaning less regulatory oversight over their contents. Although typically young people were using vaping products with nicotine concentrations below 20 mg/mL, substantial minorities reported using strengths above this legal limit or did not know the nicotine strength of products they were using. The majority of those using the higher nicotine concentration vaping products reported recently using tank devices most often and most commonly purchased them online. Doing more to restrict online sales to people under 18 years of age is therefore warranted.

Vaping among adults

Drawing on multiple surveys, smoking prevalence among adults in England in 2021 was between 12.7% and 14.9%, translating to around 6 million smokers. Based on ASH-Adult 2022 data, adult smoking prevalence in England was 13.2%. Overall, smoking has declined from around 18% in 2015. Smoking prevalence varied by age, gender and ethnicity, but notably, smoking remained more prevalent among adults from socioeconomically disadvantaged groups.

Vaping prevalence among adults in England in 2021 appeared to have increased by around one percentage point since 2020 and was around 7%, translating to just over 3 million vapers (compared to around 5% in 2015). In 2022, based on ASH-Adult data, adult vaping prevalence in England was 8.3%. Vaping was more prevalent among men than women, among people from the north of England than from Midlands or south England and among people from socioeconomically disadvantaged groups than socioeconomically advantaged groups. Similar to the findings for young people, most adults (around 99%) who had never smoked were also not currently vaping, indicating that most adults who vape had experience of smoking.

Overall, the data indicate that while smoking prevalence among adults has been steadily decreasing in the few last years, with some fluctuations, the prevalence of vaping had been stable but in the last 2 years appears to be increasing again. These trends need to

be monitored as to reach the Smokefree 2030 targets, greater use of vaping to stop smoking is likely necessary.

Other trends that need to be monitored include recent changes in the proportion of vapers who also smoke. This proportion had been declining since 2010 until 2020 but estimates from 2021 and 2022 suggest a possible increase in the proportion of current vapers who also smoke and a decline in the proportion of current vapers who are former smokers; these changes should be explored further to identify whether they are associated. In addition, data indicate a continuing increase in long-term vapers up to 2021. Between 2017 to 2021 the proportion of adult vapers who have vaped for more than 3 years has nearly doubled. Potential increasing numbers of 'dual users' and long-term vapers underscore that some areas are currently under-researched, and further effort to increase support for concurrent users to stop smoking and ensure that long-term vapers do not relapse to smoking are needed.

Tank type vaping products remained the most popular among current and former adult vapers, being used by over 50% since 2016, one in 5 used modular and about one in 6 cartridge or pod vaping devices. Similar to young people, in 2022, an increase in the use of disposable vaping products has been detected both among former and current adult vapers. The overall increase in disposable product use in 2022 was most noticeable among 18 to 24 and 25 to 34 year old participants. Nevertheless, the continuing overall popularity of tank type vaping products among adults indicates that the WHO proposal to ban open systems would seriously restrict what vaping products adults are currently using, and based on other exploratory research could benefit the tobacco industry. In 2021, less than 6% of people who vape reported using strengths of vaping liquids above those allowed by regulations (more than 20mg/mL), in contrast to the youth data. Up until 2021, the most popular strength of e-liquid remained at 6mg/mL (since 2016, when we first reported this) being consistently used by over a third of vapers over the years. Use of nicotine-free e-liquids had also remained fairly constant at less than 15% between 2016 and 2021.

Again, up until 2021, fruit, menthol/mint and tobacco were the most popular flavours among adult vapers, similar to young people. Flavour preferences appeared to have changed over time. In our 2015 review tobacco was the most commonly used flavour followed by fruit then menthol. Banning flavoured vaping products would again therefore seriously restrict what vaping products are available to adults.

Why people use vaping products

The most reported main reason for vaping among young people in 2021 were to 'give it a try', 'I like the flavours', 'vaping may be less harmful than smoking', and 'cut down the number of cigarettes smoked. The most reported main reason for vaping among adults in 2021 was to reduce the amount of tobacco smoked, to help them quit entirely, to stay off

smoking and because they enjoyed it. Similar to adults, the older teenagers (in the ITC Youth survey) who vape and are current or former smokers appeared to be vaping to reduce or stop smoking.

Vaping and smoking cessation

According to data from the Smoking Toolkit Study, among adult smokers, vaping products have remained the most common aid used in a quit attempt since 2013.

In stop smoking services, between April 2020 and March 2021, and similar to previous years, around one in twenty quit attempts were supported using a vaping product. However, quit attempts that involved the use of a vaping product (alone or in combination with medication) achieved self-reported 4-week success rates of 64.9%, compared with 58.6% for attempts not involving a vaping product. It is encouraging to see that in 2021, approximately 40% of stop smoking services, who responded to an annual survey by ASH now offer vaping products as part of their service, compared with 11% in 2019.

The latest evidence from the ongoing Cochrane collaboration living systematic review entitled ‘Electronic cigarettes for smoking cessation’ indicates that there is moderate-certainty evidence that vaping products with nicotine increase smoking cessation rates compared to NRT and vaping products without nicotine, and with less certainty that vaping products with nicotine increase smoking cessation rates compared with usual care or no treatment.

Having established how and why vaping products are being used by young people and adults we now turn to our review of the toxicant exposure and health risks of vaping products.

16.8 Exposure and potential health harms of vaping products

We searched the literature for relevant studies published between August 2017 and July 2021 to address 2 main questions:

1. What effect does vaping and secondhand exposure to vaping products have on biomarkers that are associated with the risks of cancer, respiratory, cardiovascular health conditions?

2. What are the effects of vaping among people with existing health conditions on disease outcomes?

We also used the systematic review to address secondary research questions:

1. What are the relative (compared with smoking) and absolute (compared with non-use of tobacco and nicotine products) health risks associated with using vaping products?

2. What is the nicotine exposure profile of vaping products compared with smoking and across different types of vaping products and what role does nicotine play in the health harms of vaping?

3. What effect does flavourings in vaping products, with or without nicotine have?

We also reviewed evidence on poisonings, fires and explosions attributed to nicotine vaping products.

We were also commissioned to include in vitro and in vivo studies as secondary sources of evidence to human studies. Multiple in vitro cell culture models, which exhibit many of the essential characteristics of human airway and other target cells, have been shown to be promising tools to identify and understand potential toxicities of vaping exposure. In vivo studies can assess the overall effect of vaping product exposure on the whole organism level and, unlike in vitro studies, enable the study of a large number of biological processes affecting deposition, translocation and metabolism in an intact organism. In vivo research using animal models can demonstrate the first early warning signs of potential adverse effects of vaping products given shorter life spans of animals tested (mostly mice or rats). However, apart from the extensive ethical issues involved in animal studies, the limitations of both in vitro and in vivo studies are discussed below.

We identified 413 studies for inclusion: 275 studies reporting data on human participants, 58 cell and 81 animal studies.

16.9 General limitations of the identified literature

On the whole, the overall risk of bias summaries indicated common methodological limitations. We describe limitations first as these greatly constrained the conclusions we could draw when addressing our research questions. Following this, we summarise the key findings of our review, and later turn to implications for practice and policy and future research in this area.

Choice of health risk assessment and biomarker

A major limitation was the inconsistent approach to assessing health risks. Not all studies included comparisons with smoking (relative risk assessments) nor comparisons with non-users (absolute risk assessments). Both assessments are important and should be routinely included in studies wherever feasible.

As it usually takes several years or decades for people who smoke to develop smoking-related diseases, early warning signs for diseases are needed. We were particularly interested in biomarkers of potential harm, such as lung function or chromosomal aberrations, which provide surrogate end points for disease and hence help to ameliorate problems with time lags for diseases to emerge. Based on the NASEM review, we noted that the literature on biomarkers of potential harm was still relatively nascent and therefore limited, so we also included literature on levels of biomarkers of exposure. Biomarkers of exposure assess internal exposure to tobacco smoke or vaping product aerosol constituents and are measured in body fluids. Additionally, given we have identified that most people who are exclusive vapers have been smokers, it is important to be able to distinguish the effects of prior smoking. Biomarkers of exposure, depending on their half-lives, can be less affected by prior exposure to smoking among people who are exclusive vapers, than biomarkers of potential harm, although this does not apply to all biomarkers of exposure (for example, metals), where distinguishing prior exposure to smoking is much more difficult. Nevertheless, some cross-over studies did not include adequate wash-out periods for toxicants, even for those with relatively short half-lives.

We encountered a wide range of biomarkers of exposure and potential harm being assessed and drawing on the extant literature, we limited our review to those recommended biomarkers for assessing smoking and the methods for assessing them (see chapter 2, tables 3 and 4). For biomarkers of potential harm, little attention was paid to their sensitivity, reliability and whether changes observed were clinically relevant and translated into chronic effects and relevant health outcomes.

Some biomarkers are sensitive to environmental exposures and other confounders and are therefore less useful for isolating the effects of vaping. It is also important to note that many biomarkers used to measure exposure to smoking would not be zero in never smokers and that these background levels are taken into account when comparing for example, people who vape and people who smoke. To assess this, for cross-sectional biomarkers of exposure studies which included comparison groups of vapers, smokers and non-users, we included graphs to compare visually biomarkers levels between vapers and non-users as percentages of smoker levels.

Choice of populations and user groups and characterising exposure

Here we group together who is studied and their level of exposure as these issues are inter-related.

Most of the literature we identified addressed the first research question (predominantly the effects of vaping on biomarkers); only 7 studies focused on the effects of vaping among people with existing health conditions on disease outcomes. Given these studies might identify any benefits or risks sooner than in studies which include largely healthy smokers, this is an important gap in the literature. A similar limitation also applied to the

animal studies. There was insufficient evidence of the effects of vaping product exposure on susceptible animal models, such as pregnant animals and those with underlying diseases.

The next main limitation of the extant research is the range of definitions of comparator groups. Definitions of vaping and smoking used for people who exclusively smoke, concurrently use tobacco and vaping products ('dual users'), exclusively vape and non-users varied widely across studies. Agreeing common definitions for these 4 groups would be an important stride forward and enable cross-study comparisons, and meta-analyses. For example, for never users we encountered a myriad of different inclusion criteria ranging from people who have never used one and/or the other product, to people smoking less than one cigarette or using less than one vaping product a month, no use in the past 30 days, and people who could be regular smokers or vapers of non-nicotine products, such as cannabis. The heterogeneity of concurrent user groups across the studies precluded any meaningful comparison of these groups so these data were presented in tables only. Scant attention was paid to relevant research considering concurrent user definitions and people with very different levels of smoking and vaping were eligible.

Relatedly, exposure periods (for example, frequency, heaviness, and duration of vaping) were also very heterogeneous. Definitions of vaping sometimes included occasional vaping which would underestimate exposure, particularly to biomarkers with shorter half-lives. Additionally, as most people who vape have been or are still smoking, studies—particularly those exploring ad libitum longer-term vaping product use—should include bio-verification of smoking status wherever feasible, and account for participants' smoking history and potential confounding of concurrent use at follow-ups. This need not require face-to-face contact, as hand-held carbon monoxide monitors which link to smartphones could be one way of achieving this. For all user groups it is important to be able to ascertain the duration of exposure.

In 2 of the 6 studies that assessed secondhand vaping exposure, subjects were exposed to atypically high levels of vaping emissions, and there was typically a lack of a secondhand smoking exposure comparator.

Disappointingly, some studies still referred to nicotine vaping behaviour as 'smoking'. Vaping is not smoking. Vaping products are a heterogeneous category of very different products and inadequate reporting of vaping product characteristics hindered interpretation of some studies. A comprehensive description of vaping products including device description, e-liquid description covering nicotine concentrations, nicotine protonation (freebase or salt), flavourings, PG/VG, etc. would allow better assessment and stronger conclusions. This limitation also applied to the animal and cell studies where it was not always possible to identify which specific component of vaping product aerosol was

principally responsible for any induced adverse effects due to a lack of accurate and reliable descriptions of chemical constituent levels and appropriate controls.

However, the critically important limitation of in vitro and in vivo studies was ensuring that studies are relevant for estimating human exposure and risks, which currently for most studies is unclear. It is difficult to ensure comparability of aerosol doses with doses typically absorbed by human vapers and a key concern is the relevance of adverse effects associated with vaping products in cell and animal models for humans. For in vitro studies, including the use of 3D cell culture models and air-liquid interface system for cell exposures allow an approach that more closely models human vaping product exposure.

Differences in animal models with respect to species, strains and genders pose several challenges in data interpretation. Mice remain a preferred rodent model due to their size, ease of maintenance and handling as well as similarities to humans in the metabolic pathway of nicotine. While the most widely used inbred C57BL6 strain is susceptible to atherosclerosis, the selection of Balb/c strain maximises the likelihood of toxicities associated with inflammatory responses, susceptibility to infection and development of airway responsiveness. Additionally, the majority of studies used only male animals which does not therefore allow generalisability to female vaping product users as it is unclear whether female animals would have exhibited the same outcomes, and further research is needed to examine gender differences in animals exposed to vaping product aerosol.

For animals, nose-only inhalation provides more targeted ingestion to the respiratory system and hence is more comparable to humans, but whole-body exposure measurements often result in skin and oral exposure due to grooming during and after exposure, introducing additional exposure routes.

Overall though there are concerns about restraint and confinement which have been demonstrated to increase stress which affect many of the biomarkers being studied for vaping effects. Defining the biologically relevant exposure dose that mimics real-world scenarios is challenging as there are both animal welfare and technical issues to consider. While several studies used the puffing regimen recommended by the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA, 3 second puff of 55mL every 30 seconds), other studies were designed to reflect a typical puffing topography based on the current vaping product use pattern or to match nicotine concentration of vaping product aerosol to tobacco cigarette smoke. Although it may not replicate the variation of human vaping behaviours, the limited daily exposures for 1 to 4 hours for 5 to 7 days per week may be a compromise approach to mimic human vaping product use while minimising restraint-induced stress during exposures. However, consecutive daily exposures would be desirable to minimise the effects of nicotine withdrawal.

Another key issue with animal studies is that unlike most humans who vape, the animals have not previously been exposed to cigarette smoke exposure. As outlined in a recent article, to do this would require models whereby animals are exposed to cigarette smoke

for a period of time first, and subsequently continue to be exposed, stop being exposed entirely or switch exposure to vaping products.

Eighteen animal studies were designed to investigate the changes associated with vaping over a period of at least 3 months with 10 of these studies exposing animals for 6 months or more. Assuming that the life span of mice is around 24 to 30 months, and the average life expectancy of a human being is 80 years, a 6-month inhalation exposure would represent 20% to 25% of animal's life, which is equivalent to a human chronic exposure of approximately 16 to 20 years. Therefore, more research is needed to provide insight into the potential long-term consequences of vaping product exposures that chronically develop in animals as they advance further in age.

Nicotine and its main metabolite cotinine have been measured in urine and serum in animals to ensure the inhaled dose is comparable to that in vapers for maximum translation into humans, although this cannot apply when nicotine is absent. However, given the variability in dilutions of aerosol by air, chamber sizes used for whole-body exposures and nicotine delivery efficiency of vaping product devices, it is challenging even to estimate systemic nicotine exposure, and a better marker to assess the level of vaping product exposure in the presence and absence of nicotine is therefore needed.

Choice of controls is also important in animal studies. In general, each individual experiment should include the control group of animals receiving filtered air to assess the effects of vaping product exposure. It is also desirable to include nicotine-free and/or non-flavoured vehicle controls to determine nicotine and/or -flavour dependent effects. Only a few studies, particularly on behaviour, compared the tested groups to vehicle controls (PG/VG aerosol) or baseline controls, but not air-controls, while examining time and concentration dependent effects following exposure to nicotine inhaled through vaping product aerosols.

Choice of study design

Another limitation is the variety of study designs included. While we recognise that RCTs, pharmacokinetic studies, other experimental studies, longitudinal and cross-sectional studies all make a unique contribution to the literature, the lack of standardisation within each category limited what conclusions we could draw.

Naturalistic longitudinal studies of people who vape compared with people who do not vape or smoke or with people who smoke will present the best evidence on absolute health risks of vaping as well as risks relative to smoking, over the long-term. Vaping products emerged in England around 2007, but vaping prevalence was only at a measurable level from around 2011 to 2012, meaning we now have about 10 years of data on their use. This may not be long enough to measure long-term impacts on health of

vaping which might also have a different trajectory over time (for example, take longer to emerge) to long-term impacts of smoking.

Given the data we report continue to show that most people who vape in England are former or current smokers, isolating any impacts on health separate to smoking is challenging and probably also requires a further comparison cohort of former smokers who do not vape. Groups should be followed up over an extended period of time. The UK study with the longest follow-up was just 2 years. Globally the longest follow-up was 5 years reported from a study of people who vaped with a diagnosis of COPD in Italy, and there were only 20 vapers at the 5-year follow-up. This is not a new phenomenon; to the best of our knowledge the longest follow up of users of NRT is 7 years. It is unclear whether the limited duration of these studies is a limitation of current funding mechanisms or some other reason.

In relation to pharmacokinetic studies, while controlled puffing conditions facilitate cross-product comparisons, more studies comparing ad libitum use conditions could better reflect real-life behaviours. Also, slightly longer-term sessions, for example, for 12 or 24 hours, may also highlight how nicotine intake patterns vary over the course of a day. Additionally, while pharmacokinetic studies employ some standardised conditions (for example, number of puffs over a specified time period), other elements are left to the user (for example, duration and volume of a puff) and these could also be standardised.

Other methodological issues

Due to the methodological heterogeneity of the included human studies that measured biomarker levels, we developed an algorithm to assess whether to conduct meta-analyses. This algorithm considered many of the study limitations discussed above. Studies needed to include at least 2 comparison groups (from people who use vaping products, smoke or do not use tobacco and nicotine products), clearly defined in terms of vaping and smoking status to enable exclusive users to be identified at baseline and follow-up where relevant. For longitudinal studies, adherence to study groups was also important. To be combined into one meta-analysis, biomarker data needed to be consistently acquired (for example, from one biosample category) and assessed (for example, using the same measurement method), from similar durations of exposure (acute, short-to-medium or long-term) and be provided in the appropriate format for meta-analysis. While having the same biomarkers of exposure measured across different biosamples (for example, urine, saliva, blood plasma) could be seen as a strength to enable data triangulation, these cannot be meta-analysed together; similarly for different biomarkers assessing the same toxicant. Additionally, we only included one set of data (that with the largest sample size) when multiple studies were published from the same dataset.

Overall study sample sizes were generally small, particularly in the experimental studies, and hence underpowered. Studies rarely published power analyses. These studies could

not therefore provide clear conclusions or be pooled for meta-analysis due to heterogeneous methodology.

16.10 Findings of our systematic review of exposure and potential health harms

First, we summarise our findings from the individual chapters on biomarkers of exposure to nicotine and potential toxicants and biomarkers of potential harm across multiple diseases (chapters 7 and 8), and nicotine and flavours (chapters 5 and 6). Given the most common causes of death from tobacco smoking are cancer, cardiovascular disease and respiratory disease, which collectively are responsible for about 99% of all tobacco caused deaths, we then summarise the evidence for these diseases presented in chapters 9 to 11. In these chapters, we first reviewed previous major reports (see chapter 2: methods) including the biological plausibility of vaping causing these diseases using evidence from prior reviews, then relevant biomarkers of exposure and biomarkers of potential harm. However, tobacco smoking also causes other diseases, and there are concerns that vaping may be associated with diseases not caused by smoking, so in a separate chapter 12 (other health outcomes) we reviewed literature that focused on vaping associations with a wider range of diseases.

Biomarkers of exposure to nicotine and potential toxicants

For biomarkers of exposure, we reported data on priority toxic constituents as identified by the World Health Organization (chapter 2, table 3) and by our expert collaborators. Biomarkers that were reasonably specific to the exposure are most useful and if they were related to a disease then could provide evidence of likely harm.

Chapter 7 of our review covered a substantial volume of research on biomarkers of human exposure to nicotine and potential toxicants that has been conducted since August 2017. These included 60 studies on exposure to nicotine and its metabolites, 32 on biomarkers of carbon monoxide, 28 on tobacco-specific nitrosamines, 23 on volatile organic compounds, 10 on other potential toxicants, 10 on metals and 6 on secondhand exposure to vaping products.

Overall, the reviewed data found statistically significantly and substantially reduced levels of exposure when using vaping products compared with smoking. Evidence on the absolute levels of exposure following vaping product use compared with non-use of tobacco or nicotine products varied due to methodological limitations described above and below. However, in general, toxicant exposure was similar or higher among vapers than non-users, although at substantially lower levels than when comparing smoking and non-use. The graphs showing vaper and non-user levels as a proportion of smoker levels

indicated that for many biomarkers of exposure, there was significant background/environmental exposure which needed to be taken into account.

Reviewed evidence on secondhand exposure to vaping products showed that after atypical overexposure non-users demonstrated detectable biomarker levels of potential toxicants, but biomarkers of toxicants are usually non-detectable in shorter exposure situations.

Biomarkers of potential harm to health

The biomarkers of potential harm we focused on were identified using findings from a US Food and Drug Administration sponsored 2016 workshop on biomarkers of potential harm associated with tobacco and nicotine products, and through our expert collaborators (chapter 2, table 4). Chapter 8 examined biomarkers of potential harm in humans that cut across multiple disease whereas other biomarkers of potential harm, specific to particular diseases, were discussed in the subsequent chapters on cancer, respiratory and cardiovascular diseases. In 2017, NASEM included 2 human studies that explored biomarkers of potential harm cutting across multiple diseases, whereas chapter 8 of our review included 42 studies published since August 2017. The literature on biomarkers of potential harm has therefore grown substantially since this time.

Biomarkers that cut across multiple diseases

Twenty-nine were on oxidative stress, 25 were on inflammation, 11 were on endothelial function and 4 studies reported on platelet activation. However, the included studies were methodologically heterogeneous and findings were mixed, which precluded strong conclusions. In general, there was little evidence that vaping was associated with increased oxidative stress or platelet activation biomarkers compared with smoking or not using tobacco or nicotine products; mixed findings regarding the effect of vaping on inflammation biomarkers and some evidence that endothelial function might deteriorate after acute exposure to vaping compared with no use but improves when smokers switch to vaping for a short- to medium-term period of time.

Effects of nicotine

Studies of biomarkers of exposure to nicotine generally indicated lower exposure to nicotine from short-term use but similar exposure over medium-to-longer-term duration of use. Evidence from 20 pharmacokinetic studies indicated that vaping products typically deliver lower peak and overall nicotine levels to users than smoking. Exposure to nicotine tends to increase when using e-liquids with higher nicotine concentration, nicotine salts rather than freebase nicotine, higher PG concentrations, or using tanks or modular devices with lower coil resistance or higher power settings. More experienced vapers can also have higher nicotine exposure through more effective puffing behaviour. Over time, people

who vape tend to compensate for lower nicotine concentrations by compensatory puffing which is of concern given this will increase intake of any toxicants associated with vaping. While the most popular nicotine strength is 6 mg/mL among adults who vape, it is not known whether their toxicant intake would be lower if they used higher nicotine e-liquids or whether this would mean they would vape for longer. These issues should be a focus of more research.

Previous reports indicated that vaping could result in symptoms of nicotine dependency likely to be lower than for cigarette smoking and varying by vaping product. A plethora of scales used to assess nicotine and vaping dependence make assessments of the risk and severity of vaping dependency in relation to tobacco smoking dependency difficult. A recently published review on the role of nicotine and flavour identified that vaping products with higher nicotine concentrations might increase 'abuse liability' but facilitate complete substitution of tobacco cigarettes. Flavours might interact with nicotine concentrations to affect abuse liability too.

It was very difficult to isolate nicotine effects. Only one biomarker, pulse wave velocity, seemed affected by nicotine at least in acute exposure studies. Animal and cell studies were suggestive of some adverse effects of nicotine.

Effects of flavours

Six human studies, all from the US, examined flavours. In general, positive subjective effects (for example, liking the product) for flavoured vaping products were lower than for tobacco cigarettes but higher than nicotine gum, but it was unclear whether these effects were due to nicotine delivery or consumption differences. In the few studies that assessed biomarker levels, these differed between flavours but were not tested for statistical significance. In one study, users of fruit only flavoured vaping products had significantly higher concentrations of a biomarker for acrylonitrile compared to users of a single other flavour. In one longitudinal study there seemed to be suggestive evidence that cinnamon/cinnamaldehyde containing vaping products may cause adverse reactions in some vapers.

We also identified 13 cell and 9 animal studies assessing effects of flavours. These suggested some flavourings in vaping products, particularly cinnamaldehyde and buttery/creamy flavours, have the potential to alter cellular responses but less than exposure to tobacco smoke. Exposure to unflavoured PG/VG e-liquids appeared to have little or no effect.

Cancer

To assess the effects of vaping on cancer, we summarised findings on biomarkers of exposure and biomarkers of potential harm that have relevance to cancer risk. We also

included 9 further studies on cancer-specific biomarkers related with gene expression, non-coding RNAs and DNA methylation (2 RCTs, one longitudinal study and 6 cross-sectional studies). We found that vaping generally leads to lower exposure to the many carcinogens responsible for the considerable carcinogenic effects of smoking. As the RCTs and longitudinal study included in this chapter did not have a smoking comparison group, this limits what we can infer about relative cancer risk. The cross-sectional studies, which all included people who smoked as a comparator group, reported either similar or more favourable effects of vaping than smoking on gene expression and DNA methylation. Compared with non-users, vaping was less favourable and appeared to have some unique effects, separate to smoking. As mentioned above, all the studies are limited by the possibility that other important confounders may account for the results, such as the residual effects of smoking and additional exposures that may influence cancer risk such as diet and environmental exposures.

We also included 11 human cell, one mouse cell (in vitro) and 3 animal (in vivo) studies. The majority of studies that exposed cells to an aerosol from vaping products suggest potential harm is lower or absent relative to exposure to tobacco smoke. The studies that exposed human (or in one case mouse) cells to vaping product aerosol compared to air or with no comparison group suggest cell damage from vaping aerosols, including DNA damage, reduced DNA repair activity and in some cases cell death. The animal studies point to the potential of vaping product exposure to induce DNA damage, adduct formation and carcinogenicity, but none included a comparison with smoking.

Overall, we identified a growing albeit still modest literature on how vaping may affect cancer risks in humans. Exposure to vaping generally resulted in lower levels of carcinogens compared with those found in tobacco smoke. The cell studies appear to support the human studies and suggest vaping may trigger alterations in gene expression, but at a lower extent than that observed following exposing to tobacco smoke. There were no studies that assessed the effect of vaping in people with an existing or past cancer diagnosis. Nor were we able to identify the vaping prevalence of people with cancer in England or the wider UK. These gaps in the literature need to be addressed.

Respiratory disease

To assess the effects of vaping on respiratory disease, we summarised findings on biomarkers of exposure and biomarkers of potential harm that had relevance to respiratory disease risk. There was a much greater literature identified here: 25 studies which assessed biomarkers of potential harm specifically associated with respiratory disease. However, there was no consistency in what measures were studied with what groups and over what duration of exposure, making conclusions difficult. Furthermore, few studies commented on whether there was any clinical significance of their findings.

In general, biomarkers of exposure relevant to respiratory disease were significantly lower among vapers than smokers, and in some instances reduced to levels similar to non-users (for example, carbon monoxide, most volatile organic compounds relevant to respiratory disease). This indicates that, compared with smoking, vaping exposes users to far less respiratory irritants. Studies reported similar levels of nicotine to smoking, indicating that if there are any potential risks of nicotine to respiratory disease, then they are likely present in vaping.

Findings for biomarkers of potential harm were more mixed and the methodological inconsistencies precluded conclusions being drawn. The one long-term cohort study of people with COPD diagnosis who switched from smoking to vaping at baseline and were followed up for 5 years had promising outcomes, particularly among those who switched to exclusive vaping who had higher spirometry measures than people who continued to smoke. However, given the small sample sizes (20 vaped exclusively and 19 continued to smoke), larger studies are needed to confirm these findings.

We also identified 47 cell studies that identified some adverse effects in the airway cell models linked to vaping product exposure with most frequently reported outcomes being increased cytotoxicity, enhanced markers of inflammation and oxidative stress, transcriptomic alterations and changes in cell structure and function. Where comparisons were made with tobacco smoke exposure, frequently less effects were found for vaping product exposure. Additionally, 25 animal studies were identified, 18 with mice, 5 with rats and one each in guinea pigs and sheep; only 2 studies performed nose-only inhalation exposure with the rest being whole body exposure. These studies supported the findings from cell studies that vaping product aerosol may induce inflammatory and oxidative stress responses in the airways, which was accompanied by alterations in lung function and increased airway hyper-responsiveness. Where comparisons were made with cigarette smoke exposure, findings were inconsistent. Studies utilised multiple cell and animal models, different device characteristics and exposure methods, so it is not possible to identify which constituents of the aerosol were playing a role in the reported effects.

Cardiovascular disease

To assess the effects of vaping on cardiovascular disease, we summarised findings on biomarkers of exposure and biomarkers of potential harm that had relevance to cardiovascular disease risk.

As mentioned above, studies assessing biomarkers of exposure indicated that people who vape can achieve similar levels of nicotine as people who smoke, meaning that any cardiovascular effects directly attributable to nicotine would be expected to be similar. For carbon monoxide, most volatile organic compounds relevant to cardiovascular disease and for metals, use of vaping products appears to be associated with substantially reduced

exposure compared with smoking and often similar to non-use of tobacco or vaping products.

For biomarkers of potential harm that are relevant to multiple diseases (oxidative stress, inflammation, endothelial function, platelet function), evidence synthesis was limited by heterogeneity of studies and lack of control of confounders with some suggestion that vaping was associated with substantially reduced levels of biomarkers than smoking and closer to non-use.

We identified 41 studies in humans that assessed biomarkers specific to cardiovascular health, most of them assessed heart rate or blood pressure. Vaping increased heart rate after acute exposure, with no consistent effects on blood pressure. Where people had vaped for longer periods of time, heart rate and blood pressure were lower than among people who smoked; compared with people who did not vape or smoke, findings were inconsistent, suggesting differences may be small.

Two cell studies were identified investigating the effects of vaping product exposure on cardiovascular function, both with human cells and one additionally with mice. Sixteen animal studies were also included (13 mice, 3 rats). Studies indicated adverse effects of vaping exposure, potentially less than for smoking where comparisons were included.

There were no studies that assessed effects of vaping in people with pre-existing cardiovascular diseases and there were only 2 small studies with methodological weaknesses that assessed any effects of secondhand exposure.

Other diseases

To address health outcomes beyond the main causes of smoking-related death and disease, we identified 29 studies in humans, 31 in animals and one in cells. Evidence was limited by methodological weaknesses but may indicate that vaping was associated with less favourable health than non-use but with better health than smoking. Oral or dental health has been researched more extensively than other areas and important areas such as pregnancy outcomes remain under-researched. Good quality studies assessing a wide range of health outcomes are needed, including in people with pre-existing conditions.

Poisonings, fires and explosions

Of the 413 included studies, 44 were case studies or case series reporting on poisonings, fires and explosions associated with vaping products.

In 2021, the National Poisons Information Service received 187 enquiries relating to vaping products, with just under half involving children aged 5 years and younger, out of nearly 40 thousand calls. Two UK case reports identified intentional poisoning from vaping

products and in one of those cases the person died (in 2017). In the US, according to data from a 2020 annual report by the American Association of Poison Control Centers' National Poison Data system one person died from vaping product use but no details were available. In 20 studies internationally, the majority of participants were young children who accidentally swallowed e-liquids; almost all children recovered although there were 2 fatalities. Where exposure was intentional or unknown, there were reports of 16 deaths (outside the UK).

Similar to findings discussed in our 2018 report, there were far greater numbers of fires, injuries and fatalities caused by cigarette smoking than vaping. Between January 2017 and October 2021, according to the London Fire Rescue Service there were 5,706 fires caused by cigarettes and cigarette lighters compared with 15 fires caused by vaping products. No fire related injuries or fatalities were reported from vaping related fires, compared with 676 injuries and 46 fatalities from cigarette related fires.

Two case reports involving 4 people in the UK were identified regarding exploding vaping products. No fatalities were reported. There were 23 reports identified outside of the UK from case reports/series or data from burns/surveillance of injury centres, including one fatality. Carrying the vaping product in a trouser pocket was the most common site of explosions, similar to findings from our 2018 report. Overall, incidents of exploding vaping products can be serious but appear very rare.

Summary of potential exposure and health risks literature

In this section we pull together the above findings on biomarkers and diseases, in the context of conclusions of our previous reports.

In our 2015 report, we estimated that smokers switching to nicotine vaping products reduced their risks by some 95%, drawing on similar estimates made earlier by West and others (2014) and Nutt and others (2014). These estimates were based on the understanding that smoking harms health through repeated exposure to thousands of toxicants originating due to tobacco combustion. As nicotine vaping products are non-combustible and contain far fewer constituents and these were at lower levels than in tobacco, this implied much lower vaping health risks compared with smoking.

The Royal College of Physicians in 2016 concluded that:

> "although it is not possible to quantify the long-term health risks associated with e-cigarettes precisely, the available data suggest that they are unlikely to exceed 5% of those associated with smoked tobacco products and may be substantially lower than this figure".

The estimate was still referred to in our 2018 report, concluding that:

> "vaping poses only a small fraction of the risks of smoking and switching completely from smoking to vaping conveys substantial health benefits over continued smoking. Based on current knowledge, stating that vaping is at least 95% less harmful than smoking remains a good way to communicate the large difference in relative risk unambiguously so that more smokers are encouraged to make the switch from smoking to vaping. It should be noted that this does not mean e-cigarettes are safe".

While some commentators accepted these estimates as reflecting current knowledge, others criticised them as underestimating the risks of nicotine vaping and/or putting a too precise value on a not yet fully examined phenomenon. In the US, the NASEM report in 2018 arrived at a similar conclusion, but avoided putting an actual risk-reduction estimate, concluding that:

> "Laboratory tests of e-cigarette ingredients, in vitro toxicological tests and short-term human studies suggest that e-cigarettes are likely to be far less harmful than combustible tobacco cigarettes. However, the absolute risks of the products cannot be unambiguously determined at this time."

In our 2018 report, we also discussed different approaches for assessing the health risks of vaping. We identified that the weakest evidence emanated from animal and cell studies and from laboratory studies exploring chemical composition of e-liquid or vaping product aerosol. We identified that the strongest evidence came from human studies, particularly cohort and biomarker studies of exposure and of potential harm. However, at the time of our 2018 report, the NASEM review and the COT review, which assessed the toxicological risks from vaping products, such human studies were relatively sparse.

Over recent years, the available evidence on vaping effects on health has been growing. Our current review summarised the latest evidence including many more human studies than prior reports and consolidates and enhances the evidence available to us in previous reviews. We focused largely on biomarkers of exposure and potential harm assessed in humans. The new literature that has appeared since our 2018 report and that is included in this report does not raise any substantial new concerns. However, most studies focus on acute effects of vaping. There are people who vape, almost exclusively former smokers, who have been vaping for at least 10 years and who could be compared with former smokers who do not vape to help address issues around medium- to long-term health effects of vaping. We hope that such comparisons will become a focus of future studies.

The evidence we reviewed indicated that for some individual biomarkers (with nicotine the exception) there is indeed evidence that toxicant levels are at least 95% lower in vapers than smokers with most being close to levels in non-smokers. Intuitively this should translate to a substantial lowering of risks in relation to smoking.

As referred to above, we have previously stated that vaping poses only a small fraction of the risk of smoking and is at least 95% less harmful than smoking. Our intention was to help the public and health professionals make sense of the difference in the magnitude of risk between vaping and smoking. We are aware that summarising a complex multi-dimensional construct such as the relative risks of vaping versus smoking across a range of heterogeneous products and behaviours and assessed across multiple biomarkers can be simplistic and misinterpreted. Based on the reviewed evidence, we believe that the 'at least 95% less harmful estimate' (that is, smoking is at least 20 times more harmful to users than vaping) remains broadly accurate at least over short- and medium-term periods, but it might now be more appropriate and unifying to summarise our findings using our other firm statement: that vaping poses only a small fraction of the risks of smoking. As we have also previously stated and reiterate, this does not mean vaping is risk free, particularly for people who have never smoked.

Perceptions of absolute and relative harm and how interventions affect these

In contrast to our findings on health risks, only a minority of young people (45%) and adult current smokers and vapers (34%) accurately perceived vaping to be less harmful than smoking in 2021. Thus, most young people and adult smokers in Great Britain either do not know or mistakenly think that vaping is equally or more harmful than smoking. Harm perceptions of nicotine were also similarly inaccurate, such that 62% of adult current smokers and vapers in Great Britain did not know or perceived that at least half the harms from smoking were due to nicotine. However, 60% of young people in England perceived that vaping makes it easier to quit smoking. The majority of young people in England reported noticing education campaigns or public health messages about vaping in the past 12 months (53%) in 2021. Noticing was highest on social media and in schools.

There is a need to correct inaccurate perceptions of vaping and nicotine. We therefore undertook a systematic review to examine:

1. What interventions have been effective in changing vaping harm perceptions.

2. To what extent are vaping harm perceptions predictive of any changes in vaping and smoking behaviours.

A total of 52 articles were identified, 32 addressing research question 1 and 21 addressing research question 2 (one article addressed both). As in all previous chapters, we focused on nicotine vaping.

Interventions communicating reduced harms of vaping relative to smoking (predominantly via written information or warning labels) generally increased perceptions that vaping is less harmful than smoking; most of this evidence was from adult current or former

smokers. Communicating accurate nicotine information (also predominantly via written information or warning labels) generally increased accurate nicotine harm perceptions, with all this evidence emanating from adult current or former smokers. Communicating vaping harms mainly to deter youth vaping (for example, highlighting addiction, diseases; predominantly via written information, video games, or educational workshops) generally increased perceptions that vaping harms health and is equally or more harmful than smoking. Most of this evidence was from among young people or young adults. Studies assessing the impact on harm perceptions of an outbreak of lung injuries in the US in 2019 caused by vaping illicit THC, which was mistakenly attributed to nicotine vaping, were also included. All studies consistently found that the outbreak had increased harm perceptions of vaping including inaccurate perceptions relative to smoking, with most of this evidence from adults. The longest follow-up period was 6 months, although the majority of studies used a pre-post design with no longitudinal follow-up.

Vaping harm perceptions predicted changes in vaping behaviours, such that perceiving vaping as less harmful than smoking generally predicted subsequent initiation or increases in vaping among young people or young adults and adult smokers. At the same time, perceiving vaping as harmful generally predicted not initiating vaping. Fewer studies assessed changes in smoking, although one study found that perceiving vaping as equally or more harmful than smoking predicted smoking relapse among adult former smokers. But 3 studies among young people and young adults all found no statistically significant association between harm perceptions and subsequent smoking initiation or increases. The longest follow-up period was 3 years.

Communicating vaping harms can therefore change vaping harm perceptions, which in turn can change vaping and possibly also smoking behaviours. Accurate messages on vaping relative to smoking may reduce tobacco use and are important from an ethical standpoint so as to address the prevalent misperceptions across both young people and adults. However, risk of bias in the studies assessed was high and studies were limited by short follow-up and reliance on self-report and lack of bio-verification. Studies assessing harm perceptions as well as vaping and smoking behaviours over a longer period of time are required (whether changes in perceptions and/or behaviours are sustained). No RCTs were identified that examined the impact of interventions on vaping harm perceptions, suggesting a need for this type of study. Finally, our search would have identified studies that assessed the direct impact of communicating vaping harms on changing vaping (or smoking) behaviour, but it did not find any such studies which met our inclusion criteria. Therefore, examining the direct impact of vaping communication efforts on changing vaping or smoking behaviour remains an important avenue for future research.

More broadly, the field is also limited by a lack of standardised and validated measures of vaping and nicotine harm perceptions, and the studies identified used a range of measures including harm relative to smoking, ‘absolute’ harm, risk of developing specific diseases, and risk of addiction. Standardised and validated measures of vaping and nicotine harm

perceptions should therefore be developed. Particular consideration could be given to risk (to the user and those exposed to emissions) of cancer and to respiratory health, cardiovascular health, and other diseases to match the available evidence on the impact of vaping on these diseases.

The use of other products including heated tobacco products

Since our first report in 2015, the nicotine and tobacco market in England has diversified with the entry of electronic heated tobacco products (in 2016) and more recently nicotine pouches (in 2019). Vigilance of the use of these products is important as smokers will continue to explore alternative nicotine sources, and it is likely that nicotine-naïve young people will continue to try such products as adolescence is a period of trying different things. Indeed, a recent international study on patterns of use of non-cigarette tobacco and nicotine products indicated that considerable proportions of current smokers and recent formers smokers use a variety of non-cigarette nicotine products particularly males, younger and non-daily cigarette smokers. Of particular concern was the unexpectedly high levels of use of other combustible products by recent former smokers. Across 4 countries (Australia, Canada, UK and US), the use of non-tobacco nicotine products, but not combustibles, was highest in England which the authors postulated could be due to its regulatory structure and social acceptability.

Our survey data from 2021 indicated that just over one tenth of 16 to 19 year olds in England reported ever use of a waterpipe, 4.0% reported ever using nicotine pouches, and 5.0% reported ever using smokeless tobacco. Among adults, less than 1% had tried and still used nicotine pouches, and around 1% said they used smokeless tobacco products at least weekly.

Use of heated tobacco products (HTP) remains rare in England with less than 1% of young people and adults using HTP in 2021. A recent Cochrane review concluded that there is evidence that HTP use reduces exposure to toxicants and carcinogens compared with smoking; however, there have been no studies assessing the effectiveness of these products for smoking cessation. Research independent of manufacturers is needed to assess the safety and impact of HTPs on smoking prevalence and cessation.

Overall, the youth and adult survey data indicate no increases in the use of other products over time.

16.11 Evidence statements

In this section, we provide overall conclusions for each chapter, estimating the level of evidence for key findings from our systematic review of the health harms of vaping (studies published between 1 August 2017 and 1 July 2021) and our systematic review of vaping harm perceptions (January 2007 to 1 July 2021). Conclusions on fires and poisonings also

draw on additional data. We broadly follow the definitions of level of evidence provided by NASEM (see chapter 2, table 7). As NASEM noted, the framework is a guide, but a great deal of expert judgement, in our case by the co-authors of our report, is also involved.

It is important to note that these conclusions refer to use of nicotine vaping products (although some studies assessed nicotine-free vaping products which we distinguish where appropriate), but we did not include other substances such as CBD or THC. Additionally, some conclusions may vary depending on the device characteristics of the vaping products being used. Conclusions are generally based on human studies but where there are insufficient human data, we have included conclusions based on animal and cell studies. Conclusions also refer to acute (single use to 7 days), short to medium (8 days to 12 months) or, where data were available, long-term (more than 12 months) exposures to nicotine vaping products. We also provide conclusions based on our summary of the 2022 Cochrane review on heated tobacco products.

Survey findings on young people and adult vaping in England are summarised narratively above and at the end of the relevant chapters 3 and 4.

Chapter 5. Nicotine

There is conclusive evidence that exposure to nicotine from vaping product use is variable and depends on product characteristics (for example, device type and settings, e-liquid characteristics) and vaping behaviour.

There is conclusive evidence that under controlled conditions, acute use of vaping products results in lower exposure to nicotine in users than smoking a cigarette.

There is moderate evidence that exposure to nicotine from ad libitum vaping can be comparable to that from smoking tobacco cigarettes, particularly over the medium to long-term. For experienced adult vapers, evidence is substantial.

Based on pharmacokinetic studies, there is substantial evidence that vaping product use deliver lower peak and overall nicotine levels to users than smoking, which may translate to lower dependence risks compared with smoking.

Based on pharmacokinetic studies, there is moderate evidence that nicotine exposure from vaping increases when:

- using e-liquids with higher nicotine concentration
- using e-liquids based on nicotine salts rather than freebase nicotine
- using tank or modular type vaping devices which provide more exposure than cartridge or disposable models prevalent at the time of the research

- when used by people with longer vaping experience, as they have more effective puffing behaviour

Although it's not reviewed here, previous reports found substantial evidence that vaping can result in symptoms of nicotine dependency. Previous reports also found moderate evidence that the risk and severity of nicotine dependence for vaping is lower than for cigarette smoking and would vary by product characteristics. The pharmacokinetic studies reviewed here are consistent with findings of previous reports.

Chapter 6. Flavours in vaping products

There is limited evidence that biomarker of exposure to toxicants levels may differ slightly between flavours.

There is limited evidence from animal and cell studies of potential adverse effects of specific vaping product flavours (for example, cinnamaldehyde and buttery flavours), but less than from tobacco smoke exposure.

Chapter 7. Biomarkers of exposure to nicotine and potential toxicants

There is conclusive evidence that under typical use conditions, acute and short to medium exposure to potential toxicants from vaping is significantly lower than smoking tobacco cigarettes. For long-term exposure, evidence is insufficient.

There is substantial evidence that under typical use conditions, acute and short to medium exposure to potential toxicants from vaping is higher or similar compared with non-use of tobacco or nicotine products. For long-term exposure, evidence is limited.

There is conclusive evidence that most vaping products expose users to potential toxicants. This is based on human biomarker of potential toxicants studies rather than studies of e-liquid or aerosol composition.

There is substantial evidence that under controlled conditions, the quantity and characteristics of biomarkers of potential toxicants from vaping products is relatively consistent.

There is moderate evidence that vaping exposes users to various metals. The evidence needs to be corroborated with controlled studies accounting for participants’ past smoking history.

There is limited evidence regarding exposure levels of metals when vaping from acute to long-term periods and how these levels compare to exposure to metals when smoking or not using tobacco or nicotine products.

There is moderate evidence that acute secondhand exposure to vaping product aerosol does not result in detectable levels of nicotine or biomarkers of potential toxicants in non-users of tobacco and nicotine products.

There is limited evidence that acute overexposure to secondhand vaping product aerosol results in detectable increase of nicotine or volatile organic compounds' biomarkers in non-users of tobacco and nicotine products.

Chapter 8. Biomarkers of potential harm to health cutting across several diseases

There is insufficient evidence that acute, short to medium term and long term vaping induces oxidative stress when compared with acute, short to medium term and long term smoking or non-use of tobacco or nicotine products.

There is insufficient evidence that acute, short to medium term and long term vaping is associated with increased inflammation, when compared with acute, short to medium term and long term smoking or non-use of tobacco or nicotine products.

There is limited evidence that acute vaping is associated with similar endothelial function effects as acute smoking.

There is limited evidence that short to medium term vaping is associated with improved endothelial function, compared with short to medium term smoking.

There is insufficient evidence on how vaping is associated with endothelial function, when compared with non-use of tobacco or nicotine products. There is also no available evidence regarding long-term vaping effects on endothelial function.

There is insufficient evidence on how acute, short to medium and long term vaping is associated with platelet activation, compared with smoking or non-use of tobacco or nicotine products.

Chapter 9. Cancers

There is conclusive evidence that under typical use conditions, acute and short to medium term exposure to potential carcinogens from vaping is significantly lower than smoking tobacco cigarettes.

There is substantial evidence that under typical use conditions, acute and short to medium term exposure to potential carcinogens from vaping is significantly higher or similar to non-use of tobacco or nicotine products.

There is some but currently insufficient evidence that vaping alters gene expression and DNA methylation. It is not yet clear how much this overlaps with the alteration of gene expression and DNA methylation related to smoking. There is no available evidence on how vaping affects disease progression in people with an existing or prior cancer condition.

Chapter 10. Respiratory diseases

There is conclusive evidence that under typical use conditions, acute and short to medium exposure to most potential respiratory toxicants from vaping is significantly lower than smoking tobacco cigarettes, with substantial reductions in some biomarkers. For the respiratory toxicants that were assessed at long-term exposure, evidence is moderate.

There is moderate evidence that exposure to most respiratory toxicants from vaping is similar to non-use of tobacco or nicotine products.

There is insufficient evidence of any impact of acute secondhand vaping exposure on respiratory disease.

There is insufficient evidence (from biomarkers of potential harm cutting across multiple diseases) whether vaping is associated with respiratory disease in humans.

There is insufficient evidence from spirometry, fractional exhaled nitric oxide measures, impulse oscillometer, and bronchoscopy and imaging studies as to whether vaping has any impact on lung function after acute, short to medium and long-term exposure.

There is insufficient evidence for improvement in lung function and respiratory symptoms among adult smokers with asthma who switch to vaping completely.

There is limited evidence that vaping affects lung function among adults with asthma.

There is limited evidence for reduction of chronic obstructive pulmonary disease (COPD) exacerbations among adult smokers with COPD who switch to vaping completely and continue vaping for up to 5 years.

There is limited evidence of adverse effects of vaping product exposure on the respiratory system from animal and cell studies.

Chapter 11. Cardiovascular diseases

There is no available evidence whether vaping is associated with clinical cardiovascular outcomes (for example, coronary heart disease or stroke) and no comparisons of vaping with smoking or non-use of tobacco or nicotine products.

There is no available evidence on effects of vaping on cardiovascular disease progression and no comparisons with smoking or non-use of tobacco or nicotine products.

There is moderate evidence that shortly after acute nicotine vaping, heart rate increases less than shortly after acute smoking.

There is moderate evidence that blood pressure, particularly diastolic blood pressure, increases shortly after acute nicotine vaping and that these increases are similar to increases after acute smoking.

There is limited evidence that peripheral resistance increases shortly after acute nicotine vaping.

There is moderate evidence that people who vape longer-term (often people who used to smoke) have lower heart rate than people who smoke. Where people switch incompletely, evidence of change is more limited.

There is limited evidence that people who vape longer-term have similar heart rates to people not using tobacco or nicotine products.

There is moderate evidence that people who vape longer-term (often people who used to smoke) have lower blood pressure than people who smoke and moderate evidence that blood pressure of people who vape longer-term is similar to that of non-users of tobacco or nicotine products.

There is no available evidence on whether vaping is associated with longer-term changes to arterial stiffness, compared with smoking or non-use of tobacco or nicotine products.

There is insufficient evidence on whether nicotine vaping has any effect on oxygen saturation.

There is insufficient evidence whether acute secondhand exposure to vaping has any effects on blood pressure or heart rate.

There is no available evidence on whether acute secondhand exposure to vaping has any effects on oxygen saturation, arterial stiffness or clinical cardiovascular outcomes.

Chapter 12. Other health outcomes

There is insufficient or no available evidence on the effects of vaping compared with smoking or non-use of tobacco or nicotine products on a wide range of health outcomes, for example renal, hepatic or nervous system conditions.

There is limited evidence that vaping is detrimental to oral and dental health, compared with non-use of tobacco or nicotine products and that it is less harmful than smoking.

There is insufficient evidence whether vaping affects pregnancy outcomes, compared with smoking or non-use of tobacco or nicotine products.

Chapter 13. Poisonings, fires and explosions

There is substantial evidence that the most common cause of poisonings from vaping products reported to UK and international poison centres is accidental ingestion of e-liquids by children aged 5 and younger. Intentional poisonings in adults do occur but in much smaller numbers. In very rare cases both accidental and intentional poisonings can lead to death.

Based on data from the London Fire Brigade, fires caused by vaping products are substantially less common than those caused by cigarettes.

There is substantial evidence that in very rare occasions vaping products can explode and cause serious injury requiring intensive and prolonged medical care. Explosions may be caused by malfunctioning devices and their storage while being carried.

Chapter 14. Heated tobacco products

Conclusions from this chapter are from the Cochrane review ‘Heated tobacco products for smoking cessation and reducing smoking prevalence’.

There is no available evidence on whether use of heated tobacco products (HTP) is associated with smoking cessation.

There is moderate evidence for improved amount of air that can be exhaled from the lungs (forced expiratory volume) after switching to HTP use, compared with continuing to smoke.

There is insufficient evidence of difference between HTP use and continued smoking or non-use for other biomarkers of potential harm.

There is insufficient evidence for differences in risk of adverse or serious adverse events between people randomised to switch to HTP, smoke cigarettes or attempt tobacco abstinence in the short-term.

Chapter 15. Harm perceptions and communications

There is moderate evidence that communicating reduced harms of vaping relative to smoking increased perceptions that vaping is less harmful than smoking, with most evidence from adult current and former smokers.

There is moderate evidence that communicating vaping harms increased perceptions that vaping harms health including perceptions that vaping is equally or more harmful than smoking, with most evidence from among young people or young adults.

There is substantial evidence that the EVALI outbreak increased harm perceptions of vaping, including inaccurate perceptions relative to smoking, with most evidence from among adults.

There is limited evidence that communicating accurate nicotine information increases accurate nicotine perceptions among adults.

There is substantial evidence that lower perceived harms of vaping, including the perception that vaping is less harmful than smoking, predicts subsequent initiation or increases in vaping, while perceiving vaping as equally or more harmful than smoking predicts not initiating vaping, among young people or young adults and adult smokers.

There is limited evidence that perceiving vaping as less harmful than smoking predicts subsequently quitting smoking or staying quit among adult smokers or former smokers.

There is insufficient evidence that perceiving vaping as harmful is associated with initiating smoking among young people or young adults.

16.12 Overall implications

Detailed implications are given in each of the respective chapters, and what follows are overall implications.

Policy and practice

Our findings that vaping carries a small fraction of the health risks of smoking and support people to stop smoking indicate that smokers should be encouraged to use vaping products (or medicinally licensed products) for stopping smoking or as alternative nicotine delivery devices to reduce the health harms of smoking. Our findings also confirm that never or long-term former smokers should be discouraged from taking up vaping (unless the person would otherwise relapse to smoking) as the degree of any long-term residual risk from vaping compared with non-use of tobacco or nicotine products remains unclear.

Accessibility of vaping products in England has been maintained, but cuts to government bodies responsible for overseeing their manufacture, constituents, compliance with regulations and accessibility are concerning. The recent increase in use of disposable vaping products among young people makes this an even greater concern, as unchecked, this emerging phenomenon could undermine the approach and regulatory framework for vaping products adopted in England. In addition to educational materials aimed at older smokers and why and how to vape for smoking cessation, educational materials are also needed for young people initiating vaping who would otherwise not have smoked as well as for those who need support in stopping smoking.

Over the last few years, our data sources have been constrained by the impacts of the COVID-19 pandemic, as some government surveys, such as the ‘Smoking, drinking and drug use among young people in England’ survey, did not go ahead. Given smoking remains the largest single cause of death and disease in England, this is disappointing. Fortunately, surveys, funded largely by the charitable sector or overseas funders, were sustained, although some methodological adaptations, as well as the impacts of COVID-19 on smoking and vaping behaviours itself, mean that caution must be applied when extrapolating from recent trends. It is vital that surveys assessing smoking and vaping are adequately resourced and maintained over time to enable long-term trends to be appropriately assessed. For example, given the prevalence of vaping it would be useful for the Adult Population Survey to include questions about nicotine vaping product use.

Overall, absolute and relative harm perceptions are not well aligned with the extant evidence and our findings indicate that these perceptions influence subsequent vaping and smoking behaviours. We also found that interventions can influence perceptions. Understanding and changing misperceptions is therefore important. In particular, there is very little evidence regarding the impact of the provision of accurate information about the relative harm of vaping compared with smoking among young people. There is also little evidence on the impact of interventions communicating the harms of vaping on vaping and smoking behaviours.

Systematic reviews are resource intensive, and as our cut-off date for searching the relevant literature for the health chapters was July 2021, new studies have since been published. For future evidence reviews of the health harms of vaping, a continual approach to updating the literature, similar to the living systematic review for e-cigarettes for smoking cessation by the Cochrane Tobacco Addiction Group would ensure relevant new evidence would be incorporated as it becomes available. This would ensure that policy makers are using the most up-to-date evidence.

Future research

Research on the health risks of vaping has increased rapidly over recent years as indicated by the volume of research presented by the NASEM report in 2017 and our

current review. However, much of the research is beleaguered by small sample sizes and inconsistent methodologies which are barriers to synthesis. Additionally, as evidenced by the quality assessments, efforts to reduce bias would make a strong contribution to the field.

Research on vaping should routinely examine the risk of vaping compared with no tobacco use (absolute risks) as well as risks of vaping compared with smoking (relative risks).

Much of the evidence on both absolute and relative health risks comes from cross-sectional studies. Naturalistic longitudinal studies with long-term follow-ups are needed to evaluate absolute and relative health effects.

In general, much more research on the absolute and relative health impacts from vaping is needed from UK populations. Additionally, we would suggest greater user engagement and involvement in research in this field. People who currently vape or smoke or have done so previously, can help to shape and design research to ensure that research questions are pertinent, for example reflecting what products are commonly used, and that participation in studies is maximised. They can also help to interpret findings and support dissemination efforts so that people who smoke and vape are appropriately reached.

We believe that there is a need for standardisation across studies exploring health risks and particularly studies involving biomarkers to improve evidence synthesis and comparisons across studies, and we list key recommendations below.

Choice of biomarkers

Agreeing a common set of biomarkers of exposure and biomarkers of potential harm for assessing absolute health risks of vaping as well as the relative risks of vaping compared with smoking.

Priority biomarkers should be relatively easy to measure, specific and sensitive. And if they are subject to confounders such as environmental and dietary effects, these should be accounted for in study design.

As some toxicants to do not have reliable biomarkers (for example, formaldehyde, acetone, ammonia), human exposure studies should be complemented with laboratory studies that provide data on levels of these toxicants in e-liquids or vaping product aerosol.

Consideration of half-lives of biomarkers of toxicant exposure throughout the study design, especially for participant inclusion/exclusion criteria and length of follow up assessments.

Consideration of the quality and predictive validity of biomarkers used to estimate health effects, for example, are short term changes in heart rate an accurate predictor of cardiovascular health?

Overall, studying changes to the respiratory system is important as these might be the first signals of potential harms or relative benefits from vaping. Thus, seeking a global consensus on what measures should be studied and over what duration of exposure and follow-up, in relation to respiratory disease is urgently needed.

Research on effects of vaping on other known tobacco related diseases is needed. Also, research is needed on high profile claims of vaping causing certain conditions such as adverse effects on developing adolescent brain.

Choice of user groups and exposures

It is important that studies assess the effects of vaping on progression and endpoints of disease among people with existing diseases.

Studies should also assess different population groups such as adolescents as well as adults, and include diverse groups in terms of gender, ethnicity, other substance use, mental health conditions and socioeconomic status.

Research should routinely include exclusive vapers, exclusive smokers and never users where feasible to discern unique effects of vaping. Given the prevalence of concurrent use of smoking and vaping, studying this group is also important. However, standardisation of definitions of concurrent use is needed so that findings can be compared. Biomonitoring should be used wherever possible to confirm tobacco and/or vaping product use or abstinence. Definitions for vaping should preclude concurrent smoking and a minimum duration of exclusive vaping should be defined. Frequency and intensity of use of vaping products also need to be assessed.

To identify the harms of vaping in the people who smoked in the past, studies are needed that compare long-term former smokers who do and do not vape. Similarly, to identify the harms of vaping in the absence of ever smoking, studies are needed comparing former smokers who vape with people who vape but have never smoked.

Vaping products are not a homogeneous category, and any effects are likely to differ with device types, nicotine concentration, e-liquid composition and user behaviours. As an example, most studies in the US used nicotine concentrations above the legal threshold in the UK and EU. Studies should report details on device characteristics. Findings should not be generalised to vaping products that have different characteristics from those studied.

While concerns focus on higher nicotine products being very addictive and hence a risk to naïve nicotine users, there is a dearth of research that has focused on what nicotine levels young people who vape use and their effect on dependence, particularly in England.

Exposure studies should consider background levels (levels among people who neither smoke nor vape) when comparing people who vape and people who smoke to achieve estimates of relative risk.

Better evidence on effects of secondhand exposure to vaping in comparison to exposure to smoking or air would be beneficial.

More real-world research on the impact of nicotine concentration limits in e-liquids on compensatory puffing behaviours is needed.

Choice of study designs

A gold standard pharmacokinetic standard protocol for vaping studies would be a welcome addition to the field. A recently published paper on approaches for meeting current regulatory recommendations discusses these and other issues in relation to subjective effect tests on abuse liability and dependence issues.

Cross-over studies need adequate washout periods in relation to the studied outcomes.

There is a need for standard exposure protocols for secondhand exposure studies.

As the science using human cells in vaping research has evolved in recent years, we would question the need to use animals in vaping research. Where in vitro and/or in vivo studies are necessary, it would be beneficial to introduce some degree of standardisation of certain aspects of exposure design to increase consistency across studies and develop a universal approach, enabling a rapid and comprehensive evaluation of toxicity of generated aerosols, preferably using different settings of vaping product devices.

RCTs and longitudinal studies should follow up participants for years as opposed to months to assess long-term vaping impacts. Also, to capture a cohort of nicotine-naïve people who take up vaping, very large initial samples should be recruited given any prospective tobacco use would complicate risk assessments leaving very small samples of ‘pure’ cases to study.

Greater transparency to reduce bias in research

Pre-registration of study protocols and analytical plans is common for RCTs, but less frequently used for cross-over, experimental and longitudinal studies.

Protocols should describe randomisation process, sample sizes and power calculations and how missing data will be handled.

More datasets which are open access to enable sub-group analyses or replication would also be extremely helpful.

In addition to methodological standardisation, a Bayesian approach to differentiate between truly non-significant results and lack of strong evidence for an effect (when data are insensitive to detect significant differences) could improve future research in this area.

Following reporting standards would improve consistency and quality assessments.

A clear statement on funders and potential financial and non-financial interests of research authors will allow assessment of conflicts of interest potentially influencing reporting.

More accurate reporting of research in academic and journal press releases is needed as the media generally use these to frame their reports.

While the extant research evidence is sufficient to promote vaping products for smoking cessation and tobacco harm reduction, maximising the potential of vaping products to reduce the widespread death, disease and health inequalities caused by tobacco smoking will be an important goal moving forward. Continued monitoring and surveillance of the vaping product market and implementing the above suggestions for research would mean that answers to the remaining questions on vaping would be more quickly available.

Appendices

Appendix 1. Table 1: risk of bias assessment of randomised controlled trials using Cochrane RoB2 tool

Study, year	Randomisation process	Deviations from intended interventions	Missing outcome data	Measurement of outcome	Selection of the reported result	Overall bias
Cobb et al., 2021 (1)	Low	Low	Low	Low	Some concerns	Some concerns
George et al., 2019 (2)	Low	Some concerns	Some concerns	Low	Some concerns	Some concerns
Hatsukami et al., 2019 (3)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Jay et al., 2020 (4)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Lucchiari et al., 2020 (5)	Low	Low	Low	Low	Some concerns	Some concerns
McEwan et al., 2021 (6)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Pulvers et al., 2020 (7)	Low	Some concerns	Low	Low	Some concerns	Some concerns
Round et al., 2019 (8)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Song et al., 2020 (9)	Some concerns	Low	Low	Low	Some concerns	Some concerns

Study, year	Randomisation process	Deviations from intended interventions	Missing outcome data	Measurement of outcome	Selection of the reported result	Overall bias
Staudt et al., 2018 (10)	Some concerns	Some concerns	Low	Low	Some concerns	Some concerns
Veldheer et al., 2019 (11)	Some concerns	Low	Some concerns	Low	Some concerns	Some concerns

Appendix 2. Table 2: risk of bias assessment of cross-over studies using Cochrane RoB2 tool

Study, year	Randomisation process	Bias arising from period and carryover effects	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Adriaens et al., 2018 (12)	Some concerns	Low	Some concerns	Low	Low	Some concerns	Some concerns
Amalia et al., 2021 (13)	Some concerns	Low	Low	Low	Low	Some concerns	Some concerns
Antoniewicz et al., 2019 (14)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Arastoo et al., 2020 (15)	Some concerns	Low	Low	Low	Low	Some concerns	Some concerns
Benowitz et al., 2020 (16)	Some concerns	Low	Low	Low	Low	Some concerns	Some concerns
Biondi-Zoccai et al., 2019 (17)	Low	Some concerns	Low	Low	Low	Low	Some concerns
out of Boulay et al., 2017 (18)	Some concerns	Low	Low	Low	Low	Some concerns	Some concerns
Chaumont et al., 2018 (19)	Low	Low	Low	Low	Low	Some concerns	Some concerns

Study, year	Randomisation process	Bias arising from period and carryover effects	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Chaumont et al., 2019 (20)	Low	Low	Low	Low	Low	Some concerns	Low
Chaumont et al., 2020 (21)	Low	Low	Some concerns	Low	Low	Low	Some concerns
Cobb et al., 2020 (22)	Low	Some concerns	Some concerns	Low	Low	Some concerns	Some concerns
Coppeta et al., 2018 (23)	Some concerns	Some concerns	Some concerns	Some concerns	Low	Some concerns	Some concerns
Cossio et al., 2019 (24)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Czoli et al., 2019 (25)	Some concerns	Some concerns	Some concerns	Low	Low	Some concerns	Some concerns
Ebajemito et al., 2020 (26)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Felicione et al., 2020 (27)	Low	Some concerns	Low	Low	Low	Some concerns	Some concerns
Franzen et al., 2018 (28)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Golden)son et al., 2020, US (29)	Some concerns	Low	Some concerns	Low	Low	Some concerns	Some concerns

Study, year	Randomisation process	Bias arising from period and carryover effects	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Goldenson et al., 2021, US (30)	Low	Some concerns	Low	Low	Low	Some concerns	Some concerns
Gonzalez et al., 2021 (31)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Haptonstall et al., 2020 (32)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Helen et al., 2020 (33)	Some concerns	Some concerns	Low	Low	Low	Some concerns	Some concerns
Hiler et al., 2017 (34)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Hiler et al., 2020 (35)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Ikonomidis et al., 2018 (36)	Some concerns	High	Low	Low	Low	Some concerns	High
Ip et al., 2020 (37)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Kerr et al., 2019 (38)	Low	Some concerns	Some concerns	Low	Low	Some concerns	Some concerns
Lappas et al., 2018 (39)	Some concerns	Some concerns	Low	Low	Low	Some concerns	Some concerns
Makri et al., 2020, (40)	Some concerns	Low	Low	Some concerns	Low	Some concerns	Some concerns

Study, year	Randomisation process	Bias arising from period and carryover effects	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Mallock et al., 2021 (41)	Some concerns	Some concerns	Low	Low	Low	Some concerns	Some concerns
Maloney et al., 2019 (42)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Maloney et al., 2020 (43)	Some concerns	Some concerns	Low	Low	Low	Some concerns	Some concerns
Mastrangeli et al., 2018 (44)	Some concerns	Low	Some concerns	Low	Low	Some concerns	Some concerns
Melstrom et al., 2018 (45)	Some concerns	Some concerns	Low	Low	Low	Some concerns	Some concerns
Mobarrez et al., 2020 (46)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Moheimani et al., 2017 (47)	Low	Low	Low	Some concerns	Low	Some concerns	Some concerns
Nocella et al., 2018 (48)	Some concerns	Low	Low	Low	Low	Some concerns	Some concerns
O'Connell et al., 2019 (49)	Some concerns	Low	Low	Low	Low	Some concerns	Some concerns

Study, year	Randomisation process	Bias arising from period and carryover effects	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Phillips-Waller et al., 2021 (50)	Some concerns	Some concerns	High	Low	Low	Some concerns	High
Phillips-Waller et al., 2021 (51)	Some concerns	Some concerns	Some concerns	Low	Low	High	High
Rüther et al., 2018 (52)	Some concerns	Low	Some concerns	High	Low	Some concerns	High
Spindle et al., 2018 (53)	Low	Low	Low	Low	Low	Some concerns	Some concerns
St. Helen et al., 2020 (33)	Some concerns	Low	Low	Low	Low	Some concerns	Some concerns
Stiles et al., 2018 (54)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Tzortzi et al., 2018 (55)	Some concerns	Some concerns	Low	Low	Low	Some concerns	Some concerns
Voos et al., 2019, US (56)	Low	Low	Low	Low	Low	Some concerns	Some concerns
Voos et al., 2020, US (57)	Low	Low	Low	Low	Low	Some concerns	Some concerns

Appendix 3. Table 3: risk of bias assessment of non-randomised longitudinal studies using ROBINS-I tool

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Akram et al., 2021, Saudi Arabia (58)	Serious	Critical	Serious	No Information	No Information	Moderate	Low	Critical risk of bias
Al-Hamoudi et al., 2020, Saudi Arabia (59)	Serious	No Information	Serious	No Information	No Information	Moderate	Low	Serious risk of bias
Al-Harthi et al., 2019, Saudi Arabia, (60)	Serious	No Information	Serious	No Information	Low	Low	Low	Serious risk of bias
Atuegwu et al., 2019, US (61)	Serious	Critical	Serious	Moderate	Moderate	Serious	Low	Critical risk of bias

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Baldassarri et al., 2018, US (62)	Low	Low	Low	Low	Low	Low	Low	Low risk of bias
Barna et al., 2019 (63)	Low	Low	Low	Moderate	Low	Low	Low	Moderate risk of bias
Beatrice et al., 2019 (64)	Serious	Low	Low	Low	Low	Low	Low	Serious risk of bias
Brozek et al., 2019 (65)	Moderate	Low	Low	Low	Low	Low	Low	Moderate risk of bias
Caponnetto et al., 2021 (66)	Moderate	Low	Low	Moderate	Low	Low	Low	Moderate risk of bias
Caporale et al., 2019 & Chatterjee et al., 2021 (67, 68)	Low	Low	Low	Low	Low	Low	Low	Low risk of bias

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Chatterjee et al., 2019 (69)	Low	Low	Low	Low	Low	Low	Moderate	Moderate risk of bias
Cioe et al., 2020 (70)	Moderate	Low	Low	Low	Low	Low	Low	Moderate risk of bias
Dawkins et al., 2018 (71)	Moderate	Low	Low	Low	Low	Low	Low	Moderate risk of bias
George et al., 2019 (2)	Low	Low	Low	Serious	Low	Low	Moderate	Serious risk of bias
Ghazali et al., 2019, Malaysia (72)	Critical	No Information	Serious	No Information	No Information	Moderate	Low	Critical risk of bias
Goniewicz et al., 2017 (73)	Moderate	Low	Low	Low	Low	Low	Low	Moderate risk of bias

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Hamad et al, 2021 (74)	Critical	Low	Low	Low	Low	Low	Low	Critical risk of bias
Hickling et al., 2019 (75)	Moderate	Low	Low	Moderate	Low	Low	Serious	Serious risk of bias
Ikonomidis et al., 2018 (36)	Serious	Low	Low	Serious	Low	Low	Low	Serious risk of bias
Ikonomidis et al., 2020 (76)	Moderate	Low	Low	Low	Low	Low	Low	Moderate risk of bias
Jacob et al., 2020 (77)	Serious	Moderate	Low	Moderate	Low	Low	Low	Serious risk of bias
Johnson et al., 2019 (78)	Moderate	Serious	Low	Low	Low	Low	Low	Serious risk of bias
Kimber et al., 2021 (79)	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate risk of bias

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Kizhakke Puliyakote et al., 2021 (80)	Moderate	Low	Serious	Moderate	Low	Low	Low	Serious risk of bias
Kotoulas et al., 2020 (81)	Low	Low	Low	Low	Low	Low	Low	Low risk of bias
Kuntic et al., 2020 (82)	Moderate	Moderate	Low	Low	Low	Low	Low	Moderate risk of bias
Landmesser et al., 2019, Germany (83)	Low	Low	Low	Low	Low	Low	Low	Low risk of bias
Lorkiewicz et al., 2019 (84)	Low	Low	Serious	Low	Low	Low	Low	Serious risk of bias
McClelland et al., 2019 (85)	Serious	Moderate	Low	Moderate	Low	Low	Low	Serious risk of bias

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
McClelland et al., 2020 (86)	Serious	Low	Low	Low	Low	Low	Serious	Serious risk of bias
McClelland et al., 2020 (87)	Serious	Low	Moderate	Serious	Low	Low	Low	Serious risk of bias
Munsamy et al., 2019 (88)	Low	Low	Moderate	Low	Moderate	Low	Low	Moderate risk of bias
Nga et al., 2020 (89)	Moderate	Serious	Low	Low	Low	Low	Low	Serious risk of bias
Polosa et al., 2017 (90)	Low	Moderate	Low	Low	Moderate	Low	Low	Moderate risk of bias
Polosa et al., 2018 (91)	Low	Low	Moderate	Moderate	Low	Low	Low	Moderate risk of bias
Polosa et al., 2020 (92)	Low	Low	Moderate	Moderate	Low	Low	Low	Moderate risk of bias

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Pulvers et al., 2018 (93)	Moderate	Low	Low	Low	Low	Low	Low	Moderate risk of bias
Quintana et al., 2019 (94)	Low	Low	Serious	Moderate	Moderate	Low	Low	Serious risk of bias
Quintana et al., 2021 (95)	Low	Low	Serious	Moderate	Moderate	Low	Low	Serious risk of bias
Rohsenow et al., 2018 (93)	Moderate	Low	Low	Low	Low	Low	Low	Moderate risk of bias
Rosenkilde Laursen et al., 2020 (96)	Moderate	Low	Low	Moderate	Low	Low	Low	Moderate risk of bias
Ruther et al., 2021 (97)	Low	Moderate	Moderate	Low	Moderate	Low	Low	Moderate risk of bias

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Scheibein et al., 2020 (98)	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate risk of bias
Soar et al., 2019 (99)	Low	Moderate	Low	Low	Low	Low	Low	Moderate risk of bias
Solinas et al., 2020 (100)	Moderate	Moderate	Low	Low	Low	Low	Low	Moderate risk of bias
Solingapuram Sai et al., 2019 (101)	Moderate	High	High	Low	Low	Low	High	High risk of bias
Staudt et al., 2018 (10)	Low	Low	Low	Low	Low	Low	Low	Low risk of bias
Vogel et al., 2019 (102)	Serious	Moderate	Low	Low	Low	Low	Low	Serious risk of bias
Walele et al., 2018 (103)	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate risk of bias

Study, year	Bias due to confounding	Bias in participants selection	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Yingst et al., 2019, US (104)	Low	Low	Low	Low	Low	Low	Moderate	Moderate risk of bias
Yingst et al., 2019, US (105)	Low	Low	Low	Low	Low	Low	Low	Low risk of bias

Appendix 4. Table 4: risk of bias assessment of cross-sectional studies using Biocross tool

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
AboElNaga et al., 2018 (106)	2	1	0	1	1	0	0	0	0	0	5
Al-Aali et al., 2018 (107)	2	1	1	0	1	0	0	1	1	1	8
AlQahtani et al., 2018 (108)	2	1	0	1	1	0	0	1	0	1	7
Alqahtani et al., 2019 (109)	2	1	1	1	1	2	0	1	1	0	10
Andersen et al., 2021 (110)	2	1	0	1	1	1	1	1	1	1	10
ArRejaie, et al., 2019 (111)	2	1	1	1	1	2	0	1	0	1	10

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Ashford et al., 2020 (112)	2	1	1	1	0	0	0	1	0	1	7
Aslan et al., 2019 (113)	1	1	0	2	2	0	0	1	1	1	9
Atuegwu et al., 2019 (114)	2	1	2	2	2	2	2	1	2	1	17
Badea et al., 2018 (115)	2	1	1	1	1	1	1	1	1	2	12
Bardellini et al., 2018 (116)	2	1	1	1	1	2	0	0	0	0	8
Bin Shabaib et al., 2019 (117)	2	1	1	1	1	0	0	1	0	0	7
Binshabaib, et al., 2019 (117)	2	1	1	1	1	2	0	1	1	0	10
Boas et al., 2017 (118)	2	2	1	1	2	2	0	1	0	1	12

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Boddu et al., 2019 (119)	1	1	0	1	1	2	0	1	1	1	9
Boykan et al,2019 (120)	1	1	1	2	1	1	1	1	1	1	11
Bustamante et al., 2018 (121)	2	2	1	2	1	2	0	2	1	2	15
Caliri et al., 2020 (122)	1	1	0	1	1	0	0	1	0	1	6
Carroll et al, 2018 (123)	2	1	1	1	2	2	1	1	1	1	13
Chaffee et al.,2019 (124)	2	1	1	2	1	2	0	1	0	1	11
Clemens et al., 2019 (125)	2	1	1	0	1	1	1	2	1	2	12
Corbett et al., 2019 (126)	2	2	0	2	1	2	0	2	1	2	16

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Coleman et al., 2021 (127)	1	1	1	1	2	1	1	2	1	1	12
Dai et al., 2020 (128)	2	1	1	2	2	1	1	2	1	2	15
Demir et al., 2020 {Demir, 2020 #628)	1	1	0	1	1	2	1	1	1	0	9
Doran et al., 2020 (129)	2	0	0	0	1	1	0	1	1	0	6
Faridoun et al., 2020 (130)	0	1	1	1	1	0	0	1	1	0	6
Fetterman et al., 2020 (131)	0	1	1	1	2	1	2	1	1	0	10
Frigerio et al., 2020 (132)	2	1	1	2	2	1	0	1	0	1	11
Fuller et al., 2018 (133)	2	1	0	2	1	1	0	1	1	0	9

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Ghosh et al., 2018 & Ghosh et al., 2019 (134, 135)	2	1	0	2	1	2	0	2	2	2	14
Goniewicz et al., 2018 (136)	2	1	1	2	2	1	0	1	1	2	13
Gonzalez-Roz et al., 2017 (137)	1	0	0	1	1	1	0	0	0	0	4
Gonzalez-Roz et al., 2021 (138)	1	0	0	2	1	1	0	1	0	1	7
Harlow, et al., 2021 (139)	2	2	1	2	2	2	2	1	1	2	17
Holmboe et al., 2020 (140)	2	1	2	2	2	2	1	2	1	2	17
Hwang et al., 2021 (141)	2	1	2	2	1	2	1	1	0	1	13

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Ibraheem et al., 2020 (142)	2	1	1	1	1	2	0	1	0	0	9
Jackson et al., 2020 (143)	1	1	2	1	1	1	0	1	1	1	10
Jain, 2019 (144)	2	1	1	1	2	1	0	2	1	2	13
Jain, 2021 (145)	1	1	1	2	2	1	0	1	0	1	10
Jain, 2021 (146)	0	1	1	1	1	1	0	0	0	1	6
Javed et al., 2017 (147)	1	1	1	0	1	2	0	0	0	0	6
Kalayci et al., 2020 (148)	1	3	0	2	2	3	0	1	0	1	13
Karaasalan et al., 2020 (149)	2	1	0	1	1	0	0	1	1	1	8

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Kaur et al., 2021 (150)	2	1	0	0	1	0	0	1	0	1	6
Keith et al., 2020 (151)	2	2	1	1	2	2	0	2	1	1	14
Kim et al., 2020 (152)	1	1	1	1	2	1	1	0	0	0	8
Kim et al., 2020 (153)	2	1	1	0	1	0	0	1	1	2	9
Kioi et al., 2018 (154)	2	1	1	1	2	2	1	2	2	1	15
Kumar Sinha et al., 2020 (155)	1	1	0	0	1	0	0	1	1	0	5
Lee et al., 2019 (156)	2	1	2	2	2	2	2	1	2	1	17
Lee et al., 2020 (157)	1	1	1	1	2	2	1	1	1	1	12

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Majid et al., 2021 (158)	1	2	0	1	2	2	1	0	1	1	11
Martinez-Sanchez et al., 2019 (159)	2	1	0	1	2	1	0	1	0	1	9
Md Isa et al., 2019 (160)	0	1	1	0	2	3	0	3	1	1	12
Meo et al., 2018 (161)	0	3	1	1	2	1	0	0	2	1	9
Mokeem,et al., 2018 (162)	2	1	1	0	1	2	0	1	0	0	8
Molino et al., 2021 (163)	1	1	0	0	2	1	0	1	1	1	8
Moon et al., 2020 (164)	2	1	1	1	0	0	1	1	2	2	11

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Oliveri et al., 2019 (165)	1	2	1	2	2	2	1	1	1	2	15
Orimoloye et al., 2019 (166)	2	1	1	1	2	0	1	0	1	1	10
Park et al., 2018 (167)	1	1	1	1	1	1	1	1	0	1	9
Perez et al., 2021 (168)	2	1	1	2	2	2	1	2	1	2	16
Piper et al., 2019 (169)	1	1	1	1	1	1	0	1	1	0	8
Podzolkov et al., 2020 (170)	2	1	0	1	1	0	0	0	0	0	5
Prokopowicz et al., 2019 (171)	2	2	1	1	2	1	1	2	1	1	14
Prokopowicz et al., 2020 (172)	2	2	1	2	2	2	0	2	0	1	14

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Rapp et al., 2020 (173)	2	1	1	2	2	1	0	2	1	1	13
Reidel et al., 2017 (174)	2	1	0	1	0	2	0	1	0	1	8
Rostron et al., 2020 (175)	1	1	1	0	1	1	0	2	1	2	10
Rubinstiein et al., 2018 (176)	1	1	1	2	1	2	0	0	0	1	9
Rudasingwa et al., 2021 (177)	1	1	0	1	2	1	1	2	2	1	12
Sahota et al., 2021 (178)	1	2	0	1	1	1	0	1	1	2	10
Sakamaki-Ching et al., 2020 (179)	2	2	0	0	0	0	0	2	1	2	9
Shahab et al., 2017 (180)	2	2	1	2	2	2	1	1	1	2	16

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Shields et al., 2020 (181)	2	2	1	1	1	2	1	2	1	1	14
Singh et al., 2019 (182)	2	2	0	1	0	2	0	1	0	0	8
Singh et al., 2020 (183)	2	1	0	0	1	0	0	1	1	1	7
Smith et al., 2019 (184)	2	1	1	0	1	1	0	1	1	2	10
Smith et al., 2020 (185)	2	2	1	2	2	2	1	1	1	2	16
Song et al., 2020 (186)	2	1	1	1	1	1	1	1	1	2	12
Tsai et al., 2019 (187)	2	1	0	1	1	1	1	1	0	1	9
Vohra, et al., 2020 (188)	2	1	1	1	1	2	0	0	0	0	8

Study, year	Study rationale	Design/ methods		Data analysis		Data interpretation		Biomarker measurement			Total (20 max)
	Hypothesis/ objective	Population selection	Study population representativeness	Study population characteristics	Stat. analysis	Interpretation and evaluation of results	Study limitations	Specimen characteristics and assay methods	Lab. measurement	Biomarker data modelling	
Wang et al., 2016 (189)	2	1	1	1	2	1	0	2	1	2	13
Wong et al., 2020 (190)	2	1	1	1	1	2	0	1	0	1	10
Xia et al., 2021 (191)	2	1	1	2	2	2	0	2	1	2	15
Ye et al., 2020 (192)	1	2	1	1	1	2	1	2	0	1	12

Notes: A score of 2 indicates all elements of the domain were present. A score of 1 indicates some but not all elements of the domain were present. A score of 0 indicates no elements of the domain were present.

Appendix 5. Table 5: study funding sources as reported in publications

Author, year, country	Funding as reported in publications
Aboelnaga et al., 2018, Egypt (106)	Nil.
Adriaens et al., 2018, Belgium (12)	This research received no external funding.
Al-Aali et al., 2018, Saudi Arabia (107)	The authors would like to thank the College of Dentistry Research Center and Deanship of Scientific Research at King Saud University, Saudi Arabia for funding this research project.
AlQahtani et al., 2018, Saudi Arabia (108)	The authors thank the College of Dentistry Research Center and Deanship of Scientific Research at King Saud University, Saudi Arabia for funding this research project.
Amalia et al., 2021, Spain (13)	This project has received funding from the European Union's Horizon 2020 research and innovation programme. BA received the support of a fellowship from "La Caixa" Foundation. The Tobacco Control Research Group at ICO- IDIBELL (BA, EF, MF, OT, MB, YC) is partly supported by the Ministry of Universities and Research, Government of Catalonia and thanks CERCA Programme Generalitat de Catalunya for the institutional support to IDIBELL. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Andersen et al., 2021, US (110)	This work was supported by The Smoke Free World Foundation; Foundation for a Smoke Free World.
Antoniewicz et al., 2019, Sweden (14)	This work was supported by the Swedish Heart and Lung Association, the Swedish Society of Medicine, the Swedish Heart–Lung Foundation and Stockholm County Council (ALF project). ML is supported by a clinical post-doctoral support from Karolinska Institutet and Stockholm County Council.

Author, year, country	Funding as reported in publications
Arastoo et al., 2020, US (15)	This work was supported by the Tobacco-Related Disease Research Program (TRDRP) and by National Center for Advancing Translational Science (NCATS) University of California, Los Angeles (UCLA) Clinical Translational Science Institute (CTSI) Grant.
Ashford et al., 2020, US (112)	This work was supported in part by pilot funding from the University of Kentucky College of Nursing, Office of the Dean.
Aslan et al., 2019, Turkey (113)	This project was funded by Hacettepe University, Scientific Research Projects Coordination Unit.
Badea et al., 2018, Romania (115)	This work was possible with partial funding obtained by M. Badea from UEFISCDI – Grant for international mobility and UTBV – Fellowship for international mobility 2017.
Baldassarri et al., 2018, US (62)	Yale Tobacco Center of Regulatory Science / National Institute on Drug Abuse / Food and Drug Administration Center for Tobacco Products, National Institute of Mental Health, National Heart, Lung, and Blood Institute. Dr. Eissenberg's effort is supported by P50DA036105.
Barna et al., 2019, Hungary (63)	NR
Beatrice et al., 2019, Italy (64)	This research received no external funding.
Benowitz et al., 2020, US (16)	This study was supported by grants from the National Institute on Drug Abuse, from the National Cancer Institute, and was carried out in part at the Clinical Research Center at Zuckerberg San Francisco General Hospital. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health (NIH) or the US Food and Drug Administration (FDA).
BinShabaib et. al., 2019, Saudi Arabia (117)	NR
Biondi-Zoccai et al., 2019, Italy (17)	This work was supported in part by grants from Sapienza University of Rome to Prof Frati and Prof De Falco, without any direct or indirect support from tobacco company.

Author, year, country	Funding as reported in publications
Boas et al., 2017, US (118)	This study was supported by the Tobacco-Related Disease Research Program (TRDRP), American Heart Association, Western States Affiliate, Grant-in-Aid, the National Institute of Environmental Health Sciences, National Institutes of Health, Training Grant in Molecular Toxicology, Irma and Norman Switzer Dean's Leadership in Health and Science Scholarship (RSM) and the UCLA Clinical and Translational Science Institute (CTSI).
Boykan et al., 2019, US (120)	Funded by an Intramural Research grant award to Dr Boykan from the Department of Pediatrics, School of Medicine, Stony Brook University.
Brozek et al., 2019, Poland (65)	The study was funded by scientific grant from the Medical University of Silesia.
Bustamante et al., 2018, US (121)	This study was supported by the National Cancer Institute of the National Institutes of Health and the Center for Tobacco Products of the Food and Drug Administration. LC−MS/MS was carried out in the Analytical Biochemistry Shared Resource of the Masonic Cancer Center, supported in part by Grant from the National Cancer Institute.
Caliri et al., 2020, US (122)	This work was supported by grants from the National Institute of Dental and Craniofacial Research of the National Institutes of Health and the University of California Tobacco-Related Disease Research Program.
Caponnetto et al., 2021, Italy (66)	The e-cigarettes used in the study were donated by the manufacturer, PAX Labs (on June 13, 2017 the company became known as JUUL Labs). At the time the research was conducted JUUL Labs were not part owned by Altria, a tobacco company. Due to the lack of availability at that time in Italy, PAX Labs (at that point renamed JUUL Labs) also agreed to supply cartridges/pods for a further 3 months after the end of the pilot to participants who expressed a wish to continue using them. No separate funding was secured for the study. Altria Group (formerly Philip Morris Companies) acquired a 35% stake in JUUL Labs on December 20, 2018 but the study was completed before Altria invested in JUUL.
Caporale et al., 2019, US & Chatterjee et al., 2021, US (67, 68)	Study supported by the National Institutes of Health and the National Heart, Lung, and Blood Institute.

Author, year, country	Funding as reported in publications
Carroll et al., 2018, US (123)	The National Institute on Drug Abuse at the National Institutes of Health supported this work.
Chaffee et al., 2019, US (124)	US National Institutes of Health and Food and Drug Administration National Institutes of Health.
Chaumont et al., 2018, Belgium (20)	This study was supported by the “Fonds Erasme pour la Recherche Médicale,”Belgium (to M. Chaumont), the “Fondation pour la Chirurgie Cardiaque,” Bel-gium (to M. Chaumont), the “Fondation Emile Saucez-René Van Poucke,”Belgium (to M. Chaumont), the “Prix Docteur & Mrs Rene Tagnon,” Belgium (toM. Chaumont), the “Fondation IRIS,” Belgium (to M. Chaumont), the “Prix del’Association André Vésale,” Belgium (to M. Chaumont), the “Fondation Drieghe-Miller,” Belgium (to M. Chaumont), a research grant from Astra Zeneca, Belgium (to P. van de Borne), the “Fonds Fruit de Deux Vies,” Belgium (to P. van deBorne), and the “Fond David and Alice Van Buuren,” Belgium (to P. van deBorne).
Chaumont et al., 2018, Belgium (19)	This study was supported by the “Fonds Erasme pour la Recherche Médicale”, Belgium (M.C.); the “Fondation pour la Chirurgie Cardiaque”, Belgium (M.C.); the “Fondation Emile Saucez-René Van Poucke”, Belgium (M.C.); the “Prix Docteur & Mrs Rene Tagnon”, Belgium (M.C.); the “Fondation IRIS”, Belgium (M.C.); the “Prix de l’Association André Vésale”, Belgium (M.C.); a research grant of Astra Zeneca, Belgium (P.v.d.B.); the “Fonds Fruit de Deux Vies’, Belgium (P.v.d.B.); the “Fond David and Alice Van Buuren”, Belgium (P.v.d.B.).
Chaumont et al., 2020, Belgium (21)	This study was supported by the “Fonds Erasme pour la RechercheMédicale,” Belgium (to M. Chaumont); the “Fonds Simone et Désiré Drieghe-Miller,” Belgium (to M. Chaumont); the “Fondation pour la Chirurgie Cardi-aque,” Belgium (to M. Chaumont); the “Fondation Emile Saucez-René VanPoucke,” Belgium (to M. Chaumont); the “Prix Docteur & Mrs Rene Tagnon,”Belgium (to M. Chaumont); the “Fondation IRIS,” Belgium (to M. Chaumont);the “Prix de l’Association André Vésale,” Belgium (to M. Chaumont); a research grant of Astra Zeneca, Belgium (to P. van de Borne); the “Fonds Fruitde Deux Vies,” Belgium (to P. van de Borne); and the “Fond David and AliceVan Buuren,” Belgium (to P. van de Borne).

Author, year, country	Funding as reported in publications
Chaumont et al., 2020, Belgium (21)	This study was supported by the “Fonds Erasme pour la RechercheMédicale,” Belgium (to M. Chaumont); the “Fonds Simone et Désiré Drieghe-Miller,” Belgium (to M. Chaumont); the “Fondation pour la Chirurgie Cardi-aque,” Belgium (to M. Chaumont); the “Fondation Emile Saucez-René VanPoucke,” Belgium (to M. Chaumont); the “Prix Docteur & Mrs Rene Tagnon,”Belgium (to M. Chaumont); the “Fondation IRIS,” Belgium (to M. Chaumont);the “Prix de l’Association André Vésale,” Belgium (to M. Chaumont); aresearch grant of Astra Zeneca, Belgium (to P. van de Borne); the “Fonds Fruitde Deux Vies,” Belgium (to P. van de Borne); and the “Fond David and AliceVan Buuren,” Belgium (to P. van de Borne).
Cioe et al., 2020, US (70)	This project was supported by internal funding from Brown University to Dr. Cioe. This work was facilitated by the Providence/ Boston Center for AIDS Research. Dr. Eissenberg’seffort was supported by the National Institute on Drug Abuse (NIDA) of the National Institutes of Health (NIH) and the Center for Tobacco Products of the U.S. Food and Drug Administration (FDA). Dr. Tidey’seffort was supported by grants from NIDA and the FDA Center for Tobacco Products.
Clemens et al., 2019 (125)	This work was supported in part, by grant funds from the National Institutes of Health (NIH) Clinical and Translational Science Award (CTSA) program (UL1TR000039 and KL2TR000063) and from the Arkansas Department of Health to the University of Arkansas at Pine Bluff Minority Research Center on Tobacco and Addictions. In addition, the work received support from the Arkansas Bioscience Institute and the Envoys, an advocacy group of the UAMS Cancer Institute Foundation.
Cobb et al., 2020, US (22)	This research was supported by an internal grant from Virginia Commonwealth University’s School of Nursing and Award from the National Cancer Institute of the National Institutes of Health and the Center for Tobacco Products of the U.S. Food and Drug Administration. Caroline O. Cobb and Thomas Eissenberg are also supported by Grant from the National Institute on Drug Abuse of the National Institutes of Health and the Center for Tobacco Products of the U.S. Food and Drug Administration.
Cobb et al., 2021, US (1)	National Institutes of Health, US Food and Drug Administration.

Author, year, country	Funding as reported in publications
Coleman et al., 2021, US (127)	This project was supported in part by Tobacco Centers of Regulatory Science (TCORS) Award from the National Institute on Drug Abuse (NIDA) and Food and Drug Administration (FDA), Centers of Biomedical Research Excellence Award from the National Institute of General Medical Sciences, and Institutional Training Grant Award from NIDA as well as a Canada Research Chair in Pharmacogenomics (Tyndale).
Coppeta et al., 2018, Italy (23)	NR
Corbett et al., 2018, US	This work was supported by the National Cancer Institute
Cossio et al., 2020, US (24)	NR
Czoli et al., 2019, Canada (25)	This research was supported by an Ontario Ministry of Health and Long-Term Care Health System Research Fund grant (awarded to DH). Additional support was provided by the Canadian Institutes of Health Research (CIHR), the Vanier Canada Graduate Scholarship (CDC), a CIHR and Public Health Agency of Canada, Applied Public Health Chair (DH), and an Ontario Institute for Cancer Research Investigator Award (GTF)
Dai et al., 2020, US (128)	Research of HD was partly supported by grant from the National Cancer Institute and FDA Center for Tobacco Products (CTP).
Dawkins et al., 2018, UK (71)	This study was support by grant from Cancer Research UK
De Jesus et al., 2020, US (193)	This project is supported with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, and the Center for Tobacco Products, Food and Drug Administration (FDA), Department of Health and Human Services, under a contract to West at (Contract Nos. HHSN271201100027C and HHSN271201600001C) and through an interagency agreement between the Center for Tobacco Products, the FDA, and the Centers for Disease Control and Prevention
Demir et al., 2020, Turkey (194)	NR. After this study was completed, the authors have performed studies using funds provided to the institution by e-cigarette companies.

Author, year, country	Funding as reported in publications
Ebajemito et al., 2020, UK (26)	The study was funded by British American Tobacco (Investments) Limited (BAT)
Faridoun et al., 2021, US (130)	NR
Felicione et al., US (27)	This work was supported by the National Institutes of Health (NIH) and the Center for Tobacco Products of the US Food and Drug Administration (FDA), as well as the Center for the Study of Tobacco Products Pilot Research Program. Support also provided by the National Institute of General Medical Sciences.
Fetterman et al., 2020, US (131)	This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health, and an American Heart Association Mentored Clinical and Population Research Award.
Franzen et al., 2018, Germany (28)	This study was totally financed by Medizinische Klinik III of the Universitaetsklinikum Schleswig-Holstein (UKSH).
Frigerio et al., 2020, Italy (132)	This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Fuller et al., 2018, US (133)	NR
George et al., 2019, United Kingdom (2)	The VESUVIUS (Vascular Effects ofRegular Cigarettes Versus Electronic Cigarette Use) trial was funded by the British Heart Foundation and supported by Immunoassay Biomarker Core Laboratory, University of Dundee, the Tayside Medical Sciences Centre, and the NHS Tayside Smoking Cessation Service.
Ghosh et al., 2018, US (134)	Supported by NIH grants. Research reported in this publication was in part supported by the NIH and the Food and Drug Administration (FDA) Center for Tobacco Products (CTP).
Ghosh et al., 2019, US (135)	This work was funded by NIH/FDA HL120100 and NIH/NHLBI HL135642. Research reported in this publication was in part supported by NIH and the US Food and Drug Administration Center for Tobacco Products. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the US Food and Drug Administration.
Goldenson et al., 2020, US (29)	The study was funded by Juul Labs, Inc.

Author, year, country	Funding as reported in publications
Goldenson et al., 2021, US (30)	This study was funded by Juul Labs, Inc.
Goniewicz et al., 2017, Poland (73)	Ministry of Science and Higher Education of Poland; National Institutes of Health (USA).
Goniewicz et al., 2018, US (136)	This study was supported with federal funds from the National Institute on Drug Abuse (NIDA), National Institutes of Health, and the Center for Tobacco Products, US Food and Drug Administration (FDA), Department of Health and Human Services, under contracts to Westat.
Gonzalez et al., 2021, (31)	This study was funded by a grant from Blue Cross Blue Shield of Michigan and by an endowment from the Portage Health Foundation, Houghton Michigan.
González-Roz et al., 2017, Spain (137)	Funding for this study was provided by the BBVA foundation. This institution had no role in the study design, collection, analysis or interpretation of the data, writing the manuscript, or the decision to submit the paper for publication
González-Roz et al., 2021, Canada (138)	This research was supported by the Spanish Ministry of Innovation, Research and Universities BES-2016-076663 (Dr. González-Roz), the Canadian Institutes of Health Research Project Grant (Dr. MacKillop), the Peter Boris Centre for Addictions Research (Dr. MacKillop), and the Peter Boris Chair in Addictions Research (Dr. MacKillop). Funding institutions had no role in the study design, collection, analysis or interpretation of the data, writing the manuscript, or the decision to submit the paper for publication.
Hamad et al., 2021, US (74)	Research reported in this publication was supported by grant from the National Cancer Institute and FDA Center for Tobacco Products (CTP) awarded to the University of Maryland
Haptonstall et al., 2020, US (32)	This work was supported by Tobacco-Related Disease Research Program Grants and by National Institutes of Health, National Center for Advancing Translational Science UCLA CTSI Grant.

Author, year, country	Funding as reported in publications
Hatsukami et al., 2020, US (3)	The research reported in this publication was supported by grants from the National Cancer Institute (DKH/PS), from the National Center for Advancing Translational Science of the National Institutes of Health, and from the National Institute of Drug Abuse (EM). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.
St. Helen et al., 2020, US (33)	This study was supported by grants from the National Institute on Drug Abuse and the National Cancer Institute, and was carried out in part at the Clinical Research Center at Zuckerberg San Francisco General Hospital.
Hickling et al., 2019, UK (75)	This work was funded by the Maudsley Charity; and supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London.
Hiler et al., 2017, US (34)	Research reported in this publication was supported by the National Institute on Drug Abuse of the National Institutes of Health.
Hiler et al., 2020, US (35)	Research reported in this publication was supported by the National Institute on Drug Abuse of the National Institutes of Health and the Center for Tobacco Products of the U.S. Food and Drug Administration.
Hwang et al., 2021, South Korea (141)	NR
Ikonomidis et al., 2018, Greece (36)	This study was supported by a grant from the Hellenic Cardiology Society and Hellenic Society of Lipidiology and Atherosclerosis.
Ikonomidis et al., 2020, Greece (76)	There was no funding for this study.
Ip et al., 2020, US (37)	This work was supported by Tobacco-Related Disease Research Program Grants and by National Center for Advancing Translational Science, University of California, Los Angeles, Clinical and Translational Science Institute Grant.
Jacob et al., 2020, US (77)	Research was supported by the National Institutes of Health and the U.S. Food and Drug Administration Center for Tobacco Products. Instruments and other laboratory resources were supported by the National Institutes of Health.
Jain 2021, US (146)	NR

Author, year, country	Funding as reported in publications
Jain, 2019, US (144)	Author declares that he received no funding from any private or public sources to conduct this research.
Jay et al., 2020, US (4)	This study was sponsored by JUUL Labs, Inc., the manufacturer of the JUUL NSPS.
Johnson et al., 2019, US (78)	This publication was supported by Grant from the National Institute for Occupational Safety and Health (NIOSH). Technical assistance was provided by the Division of Laboratory Sciences in the National Center for Environmental Health (NCEH). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Service.
Johnson et al., 2019, US (78)	This publication was supported by Grant from the National Institute for Occupational Safety and Health (NIOSH).Technical assistance was provided by the Division of Laboratory Sciences in the National Center for Environmental Health (NCEH). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Service
Karaaslan et al., 2020, Turkey (149)	The study did not receive any financial support.
Kaur et al, 2020, US (150)	This work was supported in part by the National Institutes of Health (NIH) (NIH 1R01HL135613), the National Cancer Institute of the NIH and the Food and Drug Administration (FDA) Center for Tobacco Products. HSC was also supported by NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Keith et al., 2020, US (151)	This research was supported by National Institutes of Health (NIH) grants
Kerr et al., 2019, UK (38)	This study was supported by a grant from the British Heart Foundation (Centre of Research Excellence Award).

Author, year, country	Funding as reported in publications
Kim et al., 2020, South Korea (153)	This study was based on Korea National Health and Nutritional Examination Survey, Ministry of Health and Welfare, Republic of Korea. We gratefully acknowledge the numerous investigators involved in the collection and management of data.
Kim et al., 2020, South Korea (152)	NR
Kim et al., 2020, South Korea (153)	This study was based on Korea National Health and Nutritional Examination Survey, Ministry of Health and Welfare, Republic of Korea. We gratefully acknowledge the numerous investigators involved in the collection and management of data.
Kimber et al., 2021, UK (79)	This work was funded by the University of East London through a PhD studentship award.
Kizhakke Puliyakote et al., 2021, US (80)	This work was supported by National Heart, Lung, and Blood Institute (NHLBI).
Kotoulas et al., 2020, Greece (81)	NR
Kuntic et al., 2020, Germany (82)	Vascular biology research grant from the Foundation Heart of Mainz (A.D., S.S., and T.M.); and vascular biology research grant from the Boehringer Ingelheim Foundation for the collaborative research group 'Novel and neglected cardiovascular risk factors: molecular mechanisms and therapeutic implications' to study the effects of environmental risk factors on vascular function and oxidative stress (TransMed PhD stipends of S.K., K.V.-M., and K.F.); R.H. holds a PhD stipend of the Max Planck Graduate Center with the Johannes Gutenberg University Mainz; T.M. is PI of the DZHK (German Center for Cardiovascular Research), Partner Site Rhine-Main,Mainz,Germany.
Landmesser et al., 2019, Germany (83)	This research was supported by Altria Client Services.
Lappas et al., 2018, Greece (39)	This work was funded by the Behrakis Foundation, Boston, MA.
Lee et al., 2020, South Korea (157)	NR

Author, year, country	Funding as reported in publications
Liu et al., 2020, US (195)	The study and the manuscript preparation were funded by RAI Services Company, a wholly owned subsidiary of Reynolds American Inc., which is a wholly owned subsidiary of British American Tobacco plc.
Lorkiewicz et al., 2019, US (84)	This work was supported by NIH grants.
Lucchiari et al., 2020, Italy (5)	The study was supported by a grant from Fondazione Umberto Veronesi (FUV).
Majid et al., 2021, US (158)	This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health. Dr Hamburg is supported by a grant from the American Heart Association.
Mallock et al., 2021, Germany (41)	This study was financially supported by funding of the German Federal Institute for Risk Assessment (BfR). Open Access funding enabled and organized by Projekt DEAL.
Maloney et al., 2019, US (42)	This study was supported by the National Institute on Drug Abuse of the National Institutes of Health and the Center for Tobacco Products of the U.S. Food and Drug Administration. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Food and Drug Administration.
Maloney et al., 2020, US (43)	This study was supported by the National Institute on Drug Abuse of the National Institutes of Health and the Center for Tobacco Products of the US Food and Drug Administration.
Martínez-Sánchez et al., 2019, Spain (159)	This project was co-funded by the Instituto de Salud Carlos III, Subdirección General de Evaluación, Government of Spain, co-funded by ISCIII-Subdirección General de Evaluación and by FEDER funds/European Regional Development Fund (ERDF) — a way to build Europe —. J.M. Martínez-Sánchez is supported by the Ministry of Universities and Research, Government of Catalonia. M. Fu, M. Ballbè and E. Fernández are supported by the Ministry of Universities and Research, Government of Catalonia. J.A. Pascual and R. Pérez Ortuño are supported by the Ministry of Universities and Research, Government of Catalonia. E. Fernández is supported by the Instituto de Salud Carlos III, Government of Spain, co-funded by the European Regional Development Fund (FEDER).

Author, year, country	Funding as reported in publications
Mastrangeli et al., 2018, Italy (44)	This study was supported by a research grant from the Sapienza University of Rome awarded to G. Biondi-Zoccai.
McClelland et al., 2020, US (86)	NR
McClelland et al., 2020, US (87)	This research was supported in part by the National Institutes of Health, BUILD Grant.
McClelland et al., 2021, US (85)	This work was funded in part by the NIH BUILD grant for minority scholars and the Faculty Development Research Fund of Detroit Mercy.
McEwan et al., 2021, UK (6)	This study was sponsored by British American Tobacco (Investments) Limited (BAT).
Melstrom et al., 2018, US (45)	NR
Meo et al., 2018, Saudi Arabia (161)	The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia
Mobarrez et al., 2020, Sweden (46)	This work was supported by the Swedish Heart and Lung foundation, the Swedish Heart and Lung Association, the Swedish Society of Medicine and Stockholm County Council (ALF project). ML is supported by a clinical post-doctoral support from Karolinska Institutet and Stockholm County Council.
Moheimani et al., 2017, US (47)	This study was supported by the Tobacco-Related Disease Research Program (TRDRP), American Heart Association, Western States Affiliate, Grant-in-Aid, the National Institute of Environmental Health Sciences, National Institutes of Health, Training Grant in Molecular Toxicology, Irma and Norman Switzer Dean's Leadership in Health and Science Scholarship, and the UCLA Clinical and Translational Science Institute (CTSI).
Mokeem et al., 2018, US (162)	This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors have no financial conflicts of interest to disclose. The authors extend their appreciation to the International Scientific Partnership Program ISPP at King Saud University, Riyadh, Saudi Arabia.

Author, year, country	Funding as reported in publications
Moon et al., 2020, South Korea (164)	The author(s) received no financial support for the research, authorship, and/or publication of this article.
Nga et al., 2020, Malaysia (89)	This work was supported by International Medical University under grant BDen Project.
Nocella et al., 2018, Italy (48)	NR
O'Connell et al., 2019, US (49)	This study was supported by Imperial Brands plc. Fontem Ventures B.V., the manufacturer of the investigational e-cigarettes used in this study, is a wholly owned subsidiary of Imperial Brands plc.
Oliveri et al., 2020, US (165)	The work was funded by Altria Client Services LLC.
Park et al., 2019, South Korea (167)	This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government
Perez et al., 2021, US (168)	The study was supported by R01 CA207491 (Oncken, PI) under a Minority Supplement for Dr. Perez. and NHLBI– PRIDE AIRE. Dr. C.O. has received free nicotine and placebo inhalers from Pfizer Pharmaceuticals for an NIH-funded smoking cessation study in pregnant women. The remaining authors report no conflict of interest.
Phillips-Waller et al., 2021 (50)	The study was funded by a Tobacco Advisory Group project grant, Cancer Research UK.
Phillips-Waller et al., 2021 (51)	The study was funded by a Tobacco Advisory Group project grant, Cancer Research UK.
Piper et al., 2019, US (169)	Research reported in this publication was supported by the National Cancer Institute and Food and Drug Administration's Center for Tobacco Products grant and Analytical Chemistry Resource grants. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Food and Drug Administration.
Podzolkov et al., 2020, Russia (170)	This trial was not funded by any organization.
Polosa et al., 2017, Italy (90)	This research was supported by Catania University grant no. 21040100 of "Ricerca Scientifica Finanziata dall'Ateneo di Catania"

Author, year, country	Funding as reported in publications
Polosa et al., 2018, Italy (91)	This research was supported by university grant of Ricerca Scientifica Finanziata dall'Ateneo di Catania.
Polosa et al., 2020, Italy (92)	This research was supported by university grant of 'Ricerca Scientifica Finanziata dall'Ateneo di Catania.
Prokopowicz et al., 2019, Poland (171)	This work was supported by the Institute of Occupational Medicine and Environmental Health Sosnowiec, Poland and Medical University of Silesia
Prokopowicz et al., 2020, Poland (172)	This work was supported by the Medical University of Silesia.
Pulvers et al., 2018, US (93)	This study was funded by the University of Minnesota (JSA) and California State University San Marcos (KP).
Pulvers et al., 2020, US (7)	Drs Pulvers and Nollen and Ms Rice were supported by grant from the National Institutes of Health (NIH). Drs Schmid and Ahluwalia were supported in part by grant from the NIH-funded Center of Biomedical Research Excellence (COBRE). Dr Schmid was partially supported by Institutional Development Award from the National Institute of General Medical Sciences of the NIH, which funds Advance Clinical and Translational Research (Advance-CTR).
Quintana et al., 2019 & 2021, US (94, 95)	This research was supported by funds from the California Tobacco-Related Disease Research Grants Program Office of the University of California (P. Quintana, Principal Investigator).
Rapp et al., 2020, US (173)	NR
Reidel et al., 2017, US (174)	The research reported in this publication was supported by the NIH and the Family Smoking Prevention and Tobacco Control Act. Supported by NIH/FDA grant.

Author, year, country	Funding as reported in publications
Rohsenow et al., 2018), US (196)	This work was supported by a Research Excellence Award from Brown University's Center for Alcohol and Addiction Studies. The funding source had no further role in study design, in the collection, analysis and interpretation of the data, in the writing of the report, or in the decision to submit the paper for publication. Dr. Eissenberg's effort was supported by the National Institute on Drug Abuse of the National Institutes of Health under Award Number P50DA036105. The content is solely the responsibility of the authors and does not necessarily re- present the official views of the National Institutes of Health or the Food and Drug Administration or the Brown University Center for Alcohol and Addiction Studies.
Rosenkilde Laursen et al., 2020, Denmark (96)	PExA AB Sweden lend us the PExA 1.0 instrument. PExA develops and commercializes a technology aimed for the discovery of early biomarkers in the field of respiratory medicine
Rostron et al., 2020, US (175)	This study has been supported with federal funds from the National Institute on Drug Abuse, National Institutes of Health, and the Food and Drug Administration, Department of Health and Human Services, under a contract to Westat. No funding was provided specifically for conducting the analysis, drafting the manuscript, or submitting this paper for publication
Round et al., 2019, US (8)	This study was funded by R.J. Reynolds Vapor Company through R.J. Reynolds Tobacco Company.
Rubinstein et al., 2018, US (176)	Funded by National Institutes of Health (NIH).
Rudasingwa et al., 2021, South Korea (177)	This research was supported by a fund of Research of Korea Centers for Disease Control and Prevention.
Ruther et al., 2018, Germany (52)	NR
Ruther et al., 2021, Germany (97)	The study required no external funding.
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Author, year, country	Funding as reported in publications
Singh et al., 2019, US (182)	This work was supported in part by National Institutes of Health (NIH) grants and the Food and Drug Administration (FDA) Center for Tobacco Products (CTP). In addition, it was in part supported by the National Cancer Institute of the NIH and the FDA CTP.
Singh et al., 2020, US (183)	This work was supported in part by a National Institutes of Health (NIH) Grants and by the National Cancer Institute of the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) Center for Tobacco Products (CTP).
Smith et al., 2019, US (184)	This research was supported by NCI and FDA Center for Tobacco Products (CTP).
Smith et al., US, UK & Poland (185)	This work was supported by Cancer Research UK. M.L.G. and D.M.S. work was supported in part by the U.S. National Institutes of Health's (NIH's) National Institute on Drug Abuse (NIDA) grant R01DA037446 and NIH's National Cancer Institute (NCI) and Food and Drug Administration Center for Tobacco Products (FDA CTP) grant. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, CDC, or the FDA.
Soar et al., 2018, UK (99)	None.
Solinas et al., 2020, Italy (100)	None to declare from all of the authors relevant to this work.
Solingapuram Sai et al., 2019, US (101)	This research was supported by the NIH and the American Cancer Society. Jed Rose receives grants from JUUL Labs Inc.; grants, personal fees, and a patent purchase agreement on a nicotine delivery system with Philip Morris International; grants from Altria; grants and personal fees from Intratab Labs Inc.; grants from the National Institute on Drug Abuse; and personal fees from Embera Neurotherapeutics, outside the submitted work. In addition, Jed Rose has a patent on a licensed nicotine delivery system. No other potential conflict of interest relevant to this article was reported.

Author, year, country	Funding as reported in publications
Song et al., 2020, US (9)	Research reported in this publication was supported by funding from the National Cancer Institute of the National Institutes of Health, the Food and Drug Administration Center for Tobacco Products, the National Center For Advancing Translational Sciences and from the Pelotonia Intramural Research Funds, and the Prevent Cancer Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the FDA.
Song et al., 2020, US (186)	Research reported in this publication was supported by funding from the NCI of the NIH, the FDA Center for Tobacco Products, the National Center for Advancing Translational Sciences, Pelotonia Intramural Research Funds, and the Prevent Cancer Foundation. The content is solely the responsibility of the authors and doesnot necessarily represent the official views of the NIH or the FDA. The authors thank the Genomics Shared Resource for performing the GeneChip Human Transcriptome Array 2.0, Center for Clinical and Translational Science for measuring the inflammatory cytokines, and Department of Pathology for BAL differential cell counts at The Ohio State University (OSU; Columbus, OH). The authors also thank the Genomics Shared Resource at Roswell Park Cancer Institute (Buffalo, NY) for conducting the Illumina Infinium Methylation EPIC BeadChip. The authors acknowledge the support of the Bioinformatics Shared Resource and the Biostatistics Shared Resource at OSU. The authors also thank the study participants, the staff and nurses of the OSU Clinical Research Center, and Sahar Kamel for assisting in recruiting participants. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact
Spindle et al., 2018, US (53)	Research reported in this publication was supported by the National Institute on Drug Abuse of the National Institutes of Health under Award and the Center for Tobacco Products of the U.S. Food and Drug Administration.
Spindle et al., 2018, US (53)	Research reported in this publication was supported by the National Institute on Drug Abuse of the National Institutes of Health under Award and the Center for Tobacco Products of the U.S. Food and Drug Administration.

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Stiles et al., 2018, US (54)	This study was funded by RJ Reynolds Vapor Company through its affiliate RJ Reynolds Tobacco Company.
Tsai et al., 2019, US (187)	NR
Tzortzi et al., 2018, Greece (55)	This project received funding from the European Union's Horizon 2020 Research and Innovation Programme. Esteve Fernandez was supported by the Ministry of Research and Universities, Government of Catalonia.
Veldheer et al., 2019, US (11)	This study was funded by the National Institutes of Health (NIH) and the U.S. Food and Drug Administration (FDA) under Award. The content is solely the responsibility of the authors and does not necessarily represent the views of the NIH or FDA. The project [publication] was supported by CTSA award from the National Center for Advancing Translational Sciences. Its contents are solely the responsibility of the authors and do not necessarily represent official views of the National Center for Advancing Translational Sciences or the National Institutes of Health.
Vogel et al., 2019, US (102)	All phases of this study were supported by the National Institute on Drug Abuse. Dr. Prochaska is also supported by the National Cancer Institute. Drs. Rubinstein and Vogel are also supported by the California Tobacco Related Diseases Research Program. The funding sources had no role in the study design, the collection, analysis, or interpretation of data, the writing of the report, or the decision to submit the manuscript for publication.
Voos et al., 2019, US (56)	This research was supported in part by NIH NIDA grant and NCI grant. Additional partial funding to Roswell Park Comprehensive Cancer Center and lab infrastructure grant from the National Institutes of Health.

Author, year, country	Funding as reported in publications
Voos et al., 2020, US (57)	This research was supported in part by NIH NIDA grant and NCI/FDA, and analytical chemistry resources grants.
Walele et al., 2018, UK (103)	This work was funded and supported by Fontem Ventures B.V. Imperial Brands Group plc is the parent company of Fontem Ventures B.V., the manufacturer of the EVP used in this study.
Wang et al., 2018, US (189)	This project is supported with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, and the Center for Tobacco Products, Food and Drug Administration, Department of Health and Human Services.
Wiener et al., 2020, US (198)	Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health.
Wong et al., 2020, Malaysia (190)	NR
Xia et al., 2021, US (191)	This manuscript is supported with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, and the Center for Tobacco Products, Food and Drug Administration, Department of Health and Human Services, under contract to Westat and through an interagency agreement between the FDA Center for Tobacco Products and the Centers for Disease Control and Prevention.
Ye et al., 2020, US (192)	This work was supported in part by a National Institutes of Health (NIH) Grant. Also, in part was supported by the National Cancer Institute of the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) Center for Tobacco Products.
Yingst et al., 2019, US (104)	This work was supported in part by the Penn State Hershey Cancer Institute, Penn State Social Science Research Institute, and Penn State Clinical & Translational Science Institute supported by the National Center for Advancing Translational Sciences, National Institutes of Health grant. The authors were supported by grant from the National Institute on Drug Abuse, National Institutes of Health and the Centers for Tobacco Products, US Food and Drug Administration.

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Note: NR—not reported.

Appendix 6. Table 6: a summary of in vivo (animal) studies evaluating health effects of vaping products inhalation exposure

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Cardiovascular					
Studies comparing effects of VP, TC and air exposure					
Olfert et al., 2018, US (199)	To evaluate the cardiovascular consequences of chronic VP exposure in mice	Mice/C57BL6 Female (n=12 for VP, 13 for TC, 11 for Air) 13-14 weeks old	Exposure: whole body inhalation Groups: PG/VG/N/F, TC, Air VP: eGrip OLED Joyetech 3rd gen PG/VG ratio: NR Nicotine: 18mg/ml Flavour: cappuccino VP Settings: 4.9V, 14.1W Regime: 55ml/puff, 5sec/puff every 99sec, 4x1h with 30-min intervals/day, 5d/week, for 8 months TC: 3R4F, same regime, 24cig/day	Echocardiography measurements, cardiac function, arterial stiffness, pulse wave velocity measurements from the carotid artery Histological assessment of lung tissue Body weight Urine cotinine levels	VP exposure increased arterial stiffness and lowered maximal aortic relaxation to methacholine, similarly to TC. Emphysema-associated histological and functional changes, and weight loss in TC-exposed mice only.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Mayyas et al., 2020, Jordan (200)	To compare the effects of VP with that of TC smoke on cardiac biomarkers of oxidative stress, inflammation, and fibrosis	Rats/Wistar Male (n=12 for VP and air, 15 for TC, 12 for Air) 8-9 weeks old	Exposure: whole body inhalation Groups: PG/VG/N, TC, Air VP: Mini Protank2 KangerTech PG/VG ratio: 7:3 Nicotine: 18mg/ml Flavour: none VP Settings: 1.8Ω, 5.76W Regime: 116.7ml/puff, 4s/puff with 10s interval, 2ml of solution/h (36mg nicotine), 1h/day, 6 days/week, for 4 weeks TC: Marlboro, side-stream smoke, 5-6 cig/1h, 10.9mg nicotine/cig (55-66mg/h), 1h/day, 6 days/week, for 4 weeks	Heart and body weight Cardiac biomarkers of oxidative stress: nitrites, thiobarbituric acid reactive substances (TBARS), super oxide dismutase (SOD), activities, catalase activities, and glutathione levels (GSH); Biomarkers of inflammation: endothelin-1, (ET-1), myeloperoxidase, (MPO), and C-reactive protein, (CRP); Biomarkers of fibrosis: transforming growth factor-beta (TGF-β), and matrix metalloproteinase −2, MMP-2)	Exposure to VP and TC decreased body weight gain ($p<.05$ both) (but not heart weight), while increased heart to body weight ratio ($p<.05$ both) Both VP and TC groups had increased oxidative stress cardiac inflammation markers, such as ET-1 ($p<.001$ and $p<.01$, respectively), cardiac nitrite ($p<.01$ both) and TBARS ($p<.0001$ and $p<.01$), activity of superoxide dismutase ($p<.05$ both), while MPO ($p<.01$) and GSH ($p<.05$) levels increased in VP only. No changes for cardiac CRP and catalase activity. Cardiac fibrosis was observed in both VP and TC groups ($p<.05$ both) coupled with an increase in the MMP-2 content ($p<.01$ and $p<.05$), while TGF-β beta protein was increased for VP only ($p<.05$).

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Szostak et al., 2020, Germany (201)	To examine the effects of VP aerosols or TC smoke on atherosclerosis progression, cardiovascular function, and molecular changes in the heart and aorta of mice	Mice/ApoE-/- Female (n=8-12) 12-14 weeks old	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N, PG/VG/N/F, TC, Air VP aerosol: generated via capillary aerosol generator (by PMI) PG/VG ratio: 3:7 Nicotine: 0, 36mg/mL Flavour: blended mix VP Settings: NR Regime: 3h/day with a 30min break after 1st hour and a 60min break after 2nd hour, 5days/week, for 3 and 6 months TC: 3R4F, same regime, matched nicotine concentration of	Biomarkers of exposure in blood, plasma, and urine: COHb, nicotine, cotinine and total metabolites, markers of oxidative stress and inflammation, haematology and lipoprotein profile. Assessment of atherosclerosis plaque and aortic root atherosclerotic plaque composition Heart ventricle histopathology and morphometry Transcriptomics and gene-set analysis Cardiac and vascular ultrasound analysis	VP aerosol exposure exerted smaller or no effect on the cardiovascular system compared to TC. Nicotine-related increases in pulse wave velocity and pulse propagation velocity in mice exposed to PG/VG/N and PG/VG/N/F aerosols, but smaller impact relative to that of TC exposure. The nicotine-containing VP aerosols caused an increase in isovolumic relaxation time similar to TC. There was no additional effect of flavour. Exposure to TC altered the systolic and diastolic functions of the heart, accelerated atherosclerotic plaque progression, altered lipid profiles, and caused alterations of the heart ventricle as well as aorta transcriptomes. Urinary markers of oxidative stress and inflammation were increased in animals exposed to TC, but not VP aerosols.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			35µg/L		No effects on plaque composition, heart weight, or heart morphology in all groups.
El-Mahdy et al., 2021, US (202)	To evaluate the long-term cardiovascular effects of VP aerosol compared with TC smoke exposure and the role of nicotine in this process	Mice/C57BL6 Male (n=20) 20 weeks old	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N, TC, Air VP: eVic Basic Joyetech with Tobeco Super Tank MINI PG/VG ratio: NR Nicotine: 0, 6, 24mg/ml Flavour: NR VP Settings: 25W, 2.24V, 0.2Ω Regime: 5s puff with 180s interval followed by 125s air exposure, repeated 20 times/day, 5 days/week, 16 or 60 weeks TC: 3R4F, 4 x	Body weight; heart weight/body weight ratio (HW/BW); blood pressure (systolic blood pressure, SBP, diastolic blood pressure, DBP, and mean blood pressure, MBP, pulse pressure) Echocardiography and myography Histopathology, dihydroethidium (DHE) Plasma cotinine	Both VP and TC exposures led to cardiovascular dysfunction, including a progressive elevation in SBP, DBP, MBP in a time-dependent manner (8-60 weeks). This was accompanied by induction of oxidative stress in aorta and heart and a significant decrease of endothelium-dependent and endothelium-independent relaxation in aorta (16-60 weeks), leading to induction of cardiac hypertrophy with elevated heart weight and aortic thickness in both TC and VP groups (32-60 weeks). Inhibition of weight gain was observed in all exposed animals. While VP-induced abnormalities were seen in the absence of nicotine, higher concentrations of nicotine exerted greater effect, similar to that of TC exposures.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			2cig/20min with 10min interval, 5days/week, 16 or 60 weeks		
Rao et al., 2020, US (203)	To investigate whether exposure to JUUL and previous generation VP aerosol impairs endothelial function comparably to cigarette smoke	Rats/Spragu Dawley Male and Female (n=8) 10 weeks old	Exposure: anesthetised rats were exposed via nose-only inhalation Groups: PG/VG/N (tank), PG/VG/N/F (pod), TC, Air VP: Nautilus Aspire tank, JUUL pod PG/VG ratio: 30:60 (pod); 67:33 (tank) Nicotine: 5% (59mg/ml, pod); 12mg/ml (tank) Flavour: tobacco (pod), none (tank) VP Settings: Regime: 10 cycles of 2s puffs over 5min TC: Marlboro Red, same regime	Endothelial function (FMD) Serum nicotine and cotinine levels	Impaired FMD following exposure to JUUL aerosol (8.6±1.5% pre-exposure vs. 3.6±0.8% post-exposure, p=.003), tank VP aerosol (8.8±0.8% pre-exposure vs. 5.3±0.6% post-exposure, p=.001), and TC smoke (8.8±1.4% pre-exposure vs. 5.8±1.0% post-exposure, p=.03). Non-significant differences between groups. Higher serum nicotine and cotinine levels in JUUL-exposed animals compared to VP and TC groups (p<.001).

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Studies comparing effects of VP and air exposure					
Qasim et al., 2018, US (204)	To investigate the effects of VP exposure on platelet function and thrombogenesis in mice	Mice/C57BL 6 Male (n=5-8) 10 weeks old	Exposure: whole body inhalation via e-Vape™ inhalation system Groups: PG/VG/N/F, Air VP: Smok TFV4 mini tank PG/VG ratio: 3:7 Nicotine: 18 mg/mL Flavour: menthol VP Settings: 5V, 0.4Ω Regime: 50mL/puff, 3sec/puff with 1min interval, 2x 100 puffs/d with 15min break, 5days/week, for 1 week	Assessment of haemostasis response (tail bleeding time) and thrombosis formation (occlusion time) Assessment of platelet function: cell count, secretion (dense and α granules), activation/aggregation (Akt, ERK, integrin and phosphatidylserine expression), and resistance to inhibition by prostacyclin (PGI2) Leukocyte activation Cotinine levels in plasma	VP exposure caused hyperactive state of platelets, with enhanced aggregation, dense and α granule secretion, activation of the αIIbβ3 integrin, phosphatidylserine expression, and Akt and ERK activation. VP-exposed platelets were resistant to inhibition by prostacyclin. Shortened thrombosis occlusion and bleeding times in VP-exposed animals.
Espinoza-Derout et al., 2019, US (205)	To evaluate cardiovascular and cardiac effects of VP exposure in mice	Mice/C57BL 6 ApoE-/- Male (n=5) 8 weeks old	Exposure: whole body inhalation Groups: PG/VG/F, PG/VG/N/F, Saline	Serum free fatty acids Echocardiography measurements	Mice exposed to nicotine containing VP had decreased left ventricular fractional shortening and ejection fraction compared with nnVP group and air control.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
		With and without Western diet: calories from fat (40%), carbohydrates (43%) and protein (17%)	VP: bluCig PLUS PG/VG ratio: NR Nicotine: 0, 24mg/mL Flavour: tobacco VP Settings: NR Regime: 4s/puff, 8 puffs with 25s intervals every 30min, repeated 24 times daily for 12h, for 12 weeks	Transcriptomic changes in heart Structural changes in cardiomyocytes ROS generation and mtDNA mutations Atherosclerotic lesions in the heart and proximal aorta Lipid peroxidation Cotinine levels in plasma	VP with nicotine induced ventricular transcriptomic changes in genes associated with metabolism, circadian rhythm, and inflammation. VP with nicotine also caused cardiomyocytes ultrastructural abnormalities indicative of cardiomyopathy and contractile dysfunction, increased oxidative stress, increased mitochondrial DNA mutations and increased atherosclerotic lesions.
Shi et al., 2019, US (206)	To examine the potential pathological effect of VP on cardiac function in mice	Mice/C57BL6 Male and female (n=17-21) 8-12 months old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG/N, Air VP: NR PG/VG ratio: 1:1 Nicotine: 24mg/mL Flavour: none	Body weight Echocardiographic measurements (The left ventricle diastolic dimension, systolic dimension, wall thickness, and heart rate) The fibrosis markers,	VP exposure increased heart tissue angiogenesis, increased the endothelial cell markers (CD31 and CD34) and slightly, but not significantly, increased collagen I protein expression in heart tissue. VP exposure caused inhibition of body weight gain.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			VP Settings: Regime: 10sec puff, 1 puff/min for 3h/day with 10min break every hour, for 2 weeks	collagen and α-SMA, the endothelial and angiogenesis markers (CD31 and CD34) in the heart tissue, plasma VEGF levels Plasma cotinine levels	
Hasan et al., 2020, US (207)	To investigate the detrimental effects of VP exposure and a high-fat diet (HFD) on cardiac structure and function in a mouse model of diet-induced obesity	Mice/C57BL 6 Male (n=5) 10 weeks old HFD-fed	Exposure: whole body inhalation Groups: PG/VG/F, PG/VG/N/F, Saline VP: bluCig PLUS PG/VG ratio: NR Nicotine: 0, 24mg/mL Flavour: tobacco VP Settings: NR Regime: 4s/puff, 8 puffs with 25s intervals every 30min, repeated 24 times daily for 12h, for 12 weeks	Body weight Plasma nicotine, cotinine and free fatty acid (FFA) levels Echocardiography Measurements of cardiomyocyte apoptosis and oxidative stress Ultrastructural abnormalities assessed by transmission electron microscopy	Decreased left ventricular (LV) fractional shortening, LV ejection fraction, and velocity of circumferential fiber shortening (VCF) in nicotine containing VP group vs. nnVP group and controls. Nicotine VP group had LV abnormalities, including lipid accumulation (ventricular steatosis), myofibrillar derangement and destruction, and mitochondrial hypertrophy. Also, increased oxidative stress, plasma free fatty acid levels, CM apoptosis, and inactivation of AMP-activated protein kinase and activation of its downstream target, acetyl-CoA-carboxylase.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Ramirez et al., 2020, US (208)	To characterise the impact of JUUL exposure on the cardiovascular system, particularly in the context of thrombogenesis and platelet function	Mice/C57BL6 (n=1-5) 10-12 weeks old	Exposure: whole body inhalation via e-Vape™ inhalation system Groups: PG/VG/N/F, Air VP: JUUL pods PG/VG ratio: NR Nicotine: 5% Flavour: menthol VP Settings: NR Regime: 50ml/3s puff with 25s intervals, 70puffs/day, for 2 weeks	Cotinine levels in urine Tail bleeding time and the time to occlusion in the ferric chloride in vivo thrombosis model. Peripheral blood cell/platelet counts, platelet aggregation, platelet dense granule secretion, platelets α granule secretion, integrin GPIIb/IIIa activation, and phosphatidylserine (PS) exposure	Exposure to JUUL shortened the thrombus occlusion as well as haemostasis/bleeding times, relative to control (medians of 14 vs. 200 seconds, p<.01 and 35 vs. 295 seconds, p<.001, respectively). JUUL exposure also caused hyperactivation of platelets, including induced platelet aggregation and secretion (p<.01 and p<.0001 in response to adenosine diphosphate and thrombin stimulation for both, respectively), as well as integrin GPIIb/IIIa activation and PS exposure (p<.0001 for both)
Li et al., 2021, US (209)	To investigate the mechanism of VP exposure accelerated atherosclerotic lesion development	Mice/ApoE-/- (n=5-10) 8-weeks old	Exposure: whole body inhalation using Teague system (TE-2) Groups: PG/VG/N/F, Air VP: NR PG/VG ratio: NR Nicotine: 24mg/mL	Plasma levels of inflammatory cytokines Flow cytometry Histological and Immunohistochemical assessment (TLR9, VCAM-1, macrophages) in heart and whole aorta	VP exposure induced atherosclerotic lesions and upregulated TLR9 expression in monocytes and in the atherosclerotic plaques. VP also increased oxidative mitochondria DNA lesion in circulating blood.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			Flavour: tobacco VP Settings: NR Regime: 2h/day, 5 days/week, for 16 weeks	Atherosclerotic lesions, Measurements of cytoplasmic mtDNA Lesion and plasma DNA damage, mtDNA/nDNA ratio in plasma cfDNA and cytoplasmic mtDNA TLR9 expression in human femoral artery atherosclerotic plaques	
Respiratory					
Studies comparing effects of VP, HTP, TC and air exposure					
Lavrynenko et al., 2020, Switzerland (210)	To assess how TC smoke, VP or HTP aerosols affect ceramide profile and related enzymes in different tissues in mice	Mice/ ApoE-/- N=10	Exposure: whole body inhalation Groups: this study analysed samples from PG/VG, PG/VG/N, PG/VG/N/F and Air-exposed groups from Szostak, et al. (201) and HTP, TC and Air from Phillips, et al. (211).	Analysis of ceramide panel in lung and plasma Proteomic and transcriptomic analysis of enzymes involved in ceramide metabolism	In contrast to TC exposure, no significant changes in the levels of the ceramides or functionally associated enzymes were observed following exposure to VP or HTP products independent of nicotine or flavourings.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			VP aerosol: generated via capillary aerosol generator (by PMI) PG/VG ratio: 3:7 Nicotine: 0, 36mg/mL Flavour: blended mix VP Settings: NR Regime: 3h/day, 5days/week for 6 months HTP: THS 2.2 and CHTP 1.2, 28µg nicotine/L, same regime TC: 3R4F, 28µg nicotine/L, same regime.		

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Studies comparing effects of VP, TC and air exposure					
Lee et al., 2018, US (212)	To compare biological changes in mice following inhalation exposures to TC smoke or VP aerosols	Mice/C57BL6 Female (n=6-12) 12 weeks old	Exposure: nose-only inhalation Groups: PG/VG/N, PG/VG/N/F, TC, Air VP: MarkTen PG/VG ratio: NR Nicotine: 40mg/ml Flavour: two different non menthol mixtures (detaills NR) (F1, F2) VP Settings: 3.5Ω Regime: 55±0.3ml/puff, 3s/puff every 30s, 180puffs/cartridge, 4h/day, 5days/week, for 3weeks TC: 3R4F, 2s/puff, 8 puffs/cig (matched nicotine concentration), same regime	Standard toxicological endpoints: in-life measurements, biomarkers of exposures (blood COHb), respiratory function (respiratory rate [RR], tidal volume [TV], and minute ventilation or volume [MV]) and histopathology Mechanistic molecular endpoints: inflammatory markers, lung transcriptomics and proteomics Terminal organ weights	Post-exposure clinical signs such as tremors and lethargy in TC group only, accompanied by increases in lung weight, BALF parameters and protein expression. TC group had a higher incidence of microscopic findings in the respiratory tract (epithelial erosion, focal metaplasia, inflammation, epithelial regeneration) compared to VP groups. Minimal microscopic changes were found in F2 VP group only. TC exposure triggered up-regulation of 4028 genes and down-regulation of 4601 genes, while 1750 genes and 1032 genes were up- and down-regulated in F2 VP group.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Glynos et al., 2018, Greece (213)	To assess the effects of a 3-day and a 4-week VP exposure on respiratory functional parameters and inflammatory responses in mice and to compare them to those of cigarette smoke	Mice/C57BL6 Male (n=5-10) 8-12 weeks old	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N, PG/VG/N/F, TC, Air VP: eRoll Joyetech PG/VG ratio: 1:1 Nicotine: 0, 18mg/mL Flavour: tobacco VP Settings: NR Regime: 4 x 8 puffs/min for 2min followed by 30min intervals/day, for 3 days or 4 weeks TC: 3R4F, 4 x 15 puffs with 30 min intervals/day, for 3 days or 4 weeks	BALF cellularity and protein content; markers of oxidative stress in the BALF and lung tissue (MDA and protein carbonyls); levels of proinflammatory cytokines in lung homogenates (IL-1β, IL-6, TNF-α) Lung histopathology and Muc5a immunohistochemistry Measurements of respiratory system mechanics (airway resistance, static compliance and tissue elasticity); airway hyperresponsiveness	Increased BALF cellularity, Muc5ac production, as well as BALF and lung oxidative stress markers in TC and VP exposed groups (especially in PG/VG/N/F). Elevated BALF protein content only in PG/VG/N/F. Altered tissue elasticity, static compliance, and airway resistance after 3 days only in PG/VG-exposed group, whereas after 4 weeks only the TC-exposed group adversely affected these parameters. Increased airway hyperresponsiveness similarly in the TC and PG/VG/N/F groups.
Reinikovaite et al., 2018, US (214)	To examine whether a 5-week exposure to VP aerosol or nicotine produce the same	Rats/ Sprague Dawley Male (n=8) 6 weeks old	Exposure: whole body inhalation using Teague system (TE-2) Groups: PG/VG/N/F, TC, s.c.	Lung morphology (mean alveolar airspace area) and lung vasculature (capillary vessel count) measurements; serum levels of nicotine and	Exposure to VP aerosol, TC and subcutaneous nicotine injections only caused significant lung tissue destruction as reflected by alveolar airspace enlargement and the loss of peripheral

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
	damaging effect on lung structure and vasculature as tobacco smoke in a rats		nicotine, Air VP: blu PG/VG ratio: NR Nicotine: 12mg/mL Flavour: tobacco VP Settings: NR Regime: 48mg nicotine/day, 4 h/day: 2×2 h with a 1-h interval, for 5 weeks TC: 3R4F, same regime s.c. injections of nicotine: 2x2 mg·kg−1/day	cotinine.	vasculature.
Ha et al., 2019, US (215)	Pilot study to demonstrate a murine model of TC smoke and VP aerosol exposure to characterise the inflammatory and immune	Mice/ C57BL6 (n=6)	Exposure: whole body inhalation Groups: PG/VG and PG/VG/N, TC, Air VP: NR PG/VG ratio: NR Nicotine: NR	Laryngeal cytokine levels (IL-25/17E, GM-CSF, IFNγ, MIP-3a/CCL20, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-21, IL-22, IL-28B, IL-10, IL-23, IL-12p70, IL-27, IL-13, IL-15, IL-17a, IL-17F, IL-33, IL-31, TGF-β, TNF-	IL-4 was elevated following exposure to TC and VP with nicotine compared to nnVP and control groups (p = 0.0418). No significant difference in the levels of other 27 cytokines. While statistically non-significant, TGF-β2 and TGF-β3 were up-

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
	responses in the larynx		Flavour: NR VP Settings: NR Regime: 3s puff with 20sec interval, 31min 40sec/day, 5days/week, for 16 weeks TC: 4 cig (matched serum cotinine), same regime	α, TGF-β1, TGF-β2, TGF-β3, CD40L)	regulated in TC group only, while IL-10 was supressed in both VP and nnVP groups (p>.05).
Madison et al., 2019, US (216)	To assess the in vivo effect of conventional tobacco smoke and components of VP aerosol on the homeostatic function of lipid biosynthesis and immunity in the lungs	Mice/C57BL6 Female (n=4-9) 8 weeks old	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N, TC, Air VP: Vapour Zeus PG/VG ratio: 6:4 Nicotine: 0, 33mg/mL Flavour: none VP Settings: 2.5Ω, 5V/1300mAh Regime: 3sec puffs with 20sec intervals for 6min 25sec/day (matched nicotine exposure dose with	Markers of airway inflammation and emphysema Histological evaluation of lung tissue Lipid homeostasis in lung macrophages and alveolar type II pneumocytes (ATIIs) Lipidomic changes in the BALF cells The expression of surfactant proteins	TC but not VP exposures induced lung inflammation and emphysema. VP exposure, independent of nicotine, altered lipid homeostasis and immune functions: lipid accumulation in alveolar macrophages and increased phospholipid pools in the airway; decreased expression of regulatory surfactant proteins; impaired expression of critical immune molecules and cytokines; irregular, unorganised lamellar bodies in ATIIs; downregulated innate immunity against viral

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			TC), 5 days/week, for 4 months TC: Marlboro Red, 3sec puff interrupted by 20sec for 4-5 min/cig, 4 cig/day, 5days/week, for 4 months	Innate immune functions of ATIIs and lung macrophages; immune responses and recovery from influenza A infection	pathogens in macrophages; enhanced influenza-associated lung inflammation and tissue damage.
Lechasseur et al., 2020, Canada (217)	To investigate the impact of dual exposure to VP vapours and cigarette smoke on lung homeostasis	Mice/Balb/c Female (n=10) 6-8 weeks old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG, TC, Dual use, Air VP: 7's hybrid vision, SS Choice LLC PG/VG ratio: 7:3 Nicotine: 0 mg/mL Flavour: none VP Settings: unknown Regime: 70ml/puff every 20sec for 2h/day, 5days/week, for 8 weeks	Lung function measurements Pulmonary circadian rhythm regulatory gene expression Markers of airway inflammation Cytokines and immunoglobulin M (IgM) in BALF and serum Myeloid cell frequencies	Exposure to VP aerosol (VP vs. air control) caused slight changes in lung tissue immune cell population, reduced pulmonary IgM levels, increased airway resistance, and reduced ICAM1, VCAM1 and PIGR expression in lungs. Compared with TC exposure, exposure to dual use also modified the effects on the pulmonary transcript levels of circadian regulatory gene, reduced circulating IgM levels, increased airway resistance, reduced expression of ICAM1, VCAM1, PIGR and altered immune cell population in lungs.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			TC: 3R4F, 8 puffs/cig over 2 hour, 5days/week, for 8 weeks Dual use		
Marshall et al., 2020, US (218)	To evaluate molecular biomarkers associated with pathogenesis of cigarette-induced pulmonary injury in animals chronically exposed to VP aerosol	Mice/ C57BL6 Female (n=15) 13-14 weeks old	Exposure: whole body inhalation Groups: this study analysed samples from PG/VG/N/F, TC and Air-exposed animals from (199) VP: eGrip OLED Joyetech 3rd gen PG/VG ratio: NR Nicotine: 18mg/mL Flavour: cappuccino VP Settings: 4.9V, 14.1W Regime: 5s/puff every 99s, 4x1h with 30-min intervals/day, 5days/week, for 8 months	Histopathological analysis of the lung tissues Gene and/or protein expressions of the CYP450 metabolism (CYP1A1, CYP2A5, and CYP3A11), oxidative stress (Nrf2, SOD1), epithelial-mesenchymal transition (E-cadherin and vimentin), lung pathogenesis (AhR), and survival/apoptotic pathways (p-AKT, BCL-XL, p53, p21, and CRM1)	Decreased expressions of E-cadherin and CRM1 and increased expression of CYP1A1, AhR, SOD1 and BCL-XL in VP group. Similar trend between VP and TC groups. Elevated nuclear accumulation of p53 in both alveolar and bronchiolar cells exposed to VP and TC, yet significantly higher in TC group.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			TC: 3R4F, same regime, 24cig/day	Urine cotinine levels	
Wawryk-Gawda et al., 2020, Poland (219)	To compare the impact of VP and TC on lung histopathological changes in an animal model	Rats/ Wistar Male (n=10)	Exposure: whole body inhalation Groups: PG/N, TC, Air VP: NR PG/VG ratio: PG only with water Nicotine: 12mg/mL Flavour: none VP Settings: 5.5V Regime: 5min/puff with 20min stop, 0.6ml of e-liquid/day, 5days/week, for 6 weeks TC: 10cig/day (matched nicotine dose, 210mg in 6 weeks)	The histomorphological evaluation of the lung tissues (H&E, periodic Acid-Schiff, PAS, Masson's trichrome staining, IHC and orcein stainings) to visualise the blood-air barrier, to assess the thickness of its membrane, collagen deposition, fibrosis, myofibroblasts, and to quantify blood vessels	Lung morphological alterations in both TC and VP groups, such as a collapse of parenchyma, hyperhagia, hyperplasia of type II of pneumocytes, collagen deposition and an increased number of macrophages within thickened alveolar septa. Yet, milder pathological changes in VP compared to TC group. Also, an initial elastolysis, thicker, disrupted, irregular, sparse elastic fibers were observed in both groups. Increased numbers of α-SMA positive myofibroblasts and blood vessels in both groups, but to a higher extent in TC.
Sun et al., 2021, US (220)	To examine the effects of PG and VG on VP-	Mice/B6C3F1 Female	Exposure: whole body inhalation using e~Aerosols	Body weight Biomarker of DNA	Reduced body weight gain only in TC exposed group ($p<0.05$)

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
	induced lung injury, inflammation and oxidative stress in mice	(n=15) 8 weeks old	system Groups: PG/VG, PG/VG/N, TC, Air VP: NJOY PG/VG ratio: 1:1 Nicotine: 0, 12 and 24mg/mL Flavour: none VP Settings: 3.7V Regime: 55ml/puff every 30s, 2h/day, 5days/week, for 8 weeks TC: 3R4F, 0.7mg nicotine/cig, smoke generated by Baumgartner Jaeger CSM 2070 system, 35ml/2s per puff every 60s, 2h/day, 5days/week, for 8 weeks	oxidative damage in plasma and lung (8-hydroxy-2′-deoxyguanosine, 8-OHdG, or its tautomer 8-oxodG) Biomarker of inflammation (C-reactive protein) and tissue injury (fibronectin) in plasma Histological evaluation of lung damage	In plasma, increased 8-oxodG levels in animals exposed to VP independent of nicotine concentration ($p<0.05$), with higher values obtained in nnVP group vs. VP with 24mg/ml nicotine ($p<0.05$). In lung tissues, increased 8-oxodG only in mice exposed to nnVP ($p<0.05$). Also, insignificant increase of plasma and lung 8-oxodG levels in TC group Exposure to TC, VP and nnVP increased plasma levels of fibonectin ($p<0.05$) and slightly, but not significantly, increased plasma C-reactive protein Higher total lung injury score in VP and nnVP exposed animals, but no statistically significant difference between groups
Wong et al., 2021, Switzerland (221)	To assess the impact of VP aerosol and TC exposure on	Mice/ApoE-/- Female (n=8-12)	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N,	Measurements of lung function, lung volume, pulmonary inflammation (cytokines in BALF),	Smaller impact on histopathological changes, lung inflammatory responses, lung transcriptome, lipidome and

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
	emphysematous changes, lung function, and molecular alterations in the respiratory system of ApoE−/− mice	12-14 weeks old	PG/VG/N/F, TC, Air VP aerosol: generated via capillary aerosol generator (by PMI) PG/VG ratio: 3:7 Nicotine: 0, 36mg/mL Flavour: blended mix VP Settings: NR Regime: 3h/day with a 30min break after 1st hour and a 60min break after 2nd hour, 5days/week, for 3 and 6 months TC: 3R4F, same regime, matched nicotine concentration of 35µg/L	emphysematous changes (histopathological analysis and morphometry), and underlying molecular changes, including oxidative stress and inflammatory responses (lung transcriptomics, proteomics, lipidomics and whole-genome analysis) Blood and urine levels of nicotine, its metabolites and PG	proteome dysregulation and changes in DNA methylation following VP exposure in comparison with TC exposure.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Studies comparing effects of VP and air exposure					
Kleinman et al., 2020, US (222)	To demonstrate the observed VP use-associated lung injury (EVALI)-like condition in animal model following VP exposure without the use of tetrahydrocannabinol or vitamin E	Rats N=5-18	Exposure: nose-only inhalation Groups: PG/VG/F, Air VP: NR PG/VG ratio: 1:1 Nicotine: 0 mg/mL Flavour: tobacco VP Settings: 60W, 70W Regime: a single 2h exposure using VP with nickel-chromium alloy (NC, n=18) and stainless-steel atomizer (SS, n=5)	Histological analysis	Initial findings - NC group demonstrated thickening of the alveolar wall with foci of inflammation, red blood cell congestion, obliteration of alveolar spaces, and pneumonitis (2 of 7 rats); bronchi showed accumulation of fibrin, inflammatory cells, and mucus plugs. SS group showed normal histology except for 1 mouse with small area of inflammation.
Chen et al., 2018, Australia (223)	To investigate the effect of intrauterine VP exposure in mice on the markers of lung development and inflammation of both mothers and	Mice/Balb/c Female (n=6) 7 weeks old Male offspring (n=14-20), studied at	Exposure: whole body inhalation Groups: PG/VG/F, PG/VG/N/F, Air VP: NEBOX KangerTech PG/VG ratio: 1:1 Nicotine: 0,	mRNA or protein expression of lung-developmental markers (platelet-derived growth factor, PDGF, ephrine B2, EphB2, and surfactant protein C, Sftpc); cytokines and factors involved in	Increased proinflammatory cytokines IL-1β, IL-6, and TNF-α in the lungs of mothers exposed to VP and nnVP aerosols. In the adult offspring, TNF-α was also increased, while IL-1β was decreased. Alterations in inflammatory

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
	offspring	Postnatal day 1 and 20 and at 13 weeks	18mg/mL Flavour: tobacco VP Settings: NR Regime: 4 x 5s puff at 30W with 20s interval, 2 x 15min/day, 6 weeks before gestation, during gestation and lactation	inflammatory signalling pathways (TNF-α, IL-1β, IL-6, IL-13, IL-5, IL-4, IL-6, total and phosphorylated Erk1/2, JNK, p38, p65 and NF-kB); global DNA methylation Body weight	signalling pathways in the mothers' (changes in ERK1/2 and JNK expression) and offspring's (changes in p38 and p65) lungs. Reduced body weight in in utero nicotine-exposed group at weaning (postnatal day 20). Increased DNA methylation in in utero VP exposed offspring at postnatal day 1.
Chapman et al., 2019, US (224)	To investigate the effects of favoured VP and nnVP on the development and severity of allergic airways disease in mice	Mice/Balb/c Male and Female (n=8-12 for treated and n=6-10 for controls) 8 weeks old 50µg of intranasal house dust mite or phosphate-buffered saline (Days	Exposure: whole body inhalation using Groups: PG/VG, PG/VG/N, PG/VG/F, PG/VG/N/F, Air VP: eVic-VT Joyetech PG/VG ratio: 1:1 Nicotine: 0, 12mg/ml Flavours: Black Licorice, cinnamon 'Kola' and 'Cinnacide', creamy/buttery	Assessment of airway mechanics and airway hyperresponsiveness Analysis of BALF Measurement of collagen content Histopathology	Flavoured VP with nicotine suppressed airway inflammation ($p<0.001$ for all) with no effect on airway hyperresponsiveness or airway remodeling. nnVP cinnamon flavour ('Cinnacide') reduced airway inflammation ($p=0.045$) and increased peripheral airway hyperresponsiveness ($p=0.02$). nnVP creamy/buttery flavour ('Banana Pudding') increased soluble lung collagen content ($p=0.049$). nnVP Black Licorice exaggerated

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
		0, 7, 14, 14-18)	'Banana Pudding' VP Settings: 0.4Ω Regime: 4s puff every 60s, 2x30min/day, 6 days/week, for 18 days		airway inflammation but not statistically significantly (p=0.089).
Chung et al., 2019, US (225)	To test the effects of VP exposure on airway mucociliary function in the airways of a novel, ovine large animal model	Sheep Female (n=2-3)	Exposure: administrated by nebulization or by vaping Groups: nebulised or aerosolised PG/VG/N, ethanol control VP: eVic Joyetech for vaping, Airlife for nebulisation PG/VG ratio: 1:1 in ethanol Nicotine: 10, 15 or 20 mg/ml (nebulised) or 36mg/ml (aerosolised) Flavour: none	Marker of mucociliary clearance, tracheal mucus velocity (TMV) Plasma cotinine	Nebulised VP aerosol with nicotine reduced TMV in a nicotine dose-dependent manner ($p<0.05$). The effect of VP exposure on TMV was reversed by pre-treatment with inhaled TRPA1 antagonist A967079. Better systemic nicotine delivery with nebulisation compared to vaping.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			VP Settings: NR Regime: two inhalations with 6h interval (nebulised); 60ml/4.5s/puff, 40 inhalations (aerosolised)		
Cirillo et al., 2019, Italy (226)	To investigate the effects of VP resistance on carbonyls production from non-nicotine vapour and the pulmonary oxidative and inflammatory status in rats	Rat/Sprague Dawley Male (n=10) 8 weeks old	Exposure: whole body inhalation Groups: PG/VG/F, Air VP: Eleaf Pico PG/VG ratio: 1:1 Nicotine: 0 mg/mL Flavour: 10% red fruit VP Settings: 3.5V, 0.25Ω or 1.5Ω Regime: 11 cycles of 2 puffs (6s on; 5s off; 6s on), with 20 min intervals, 5days/week, for 28 days	Carbonyl compounds in VP vapours The pulmonary inflammation, oxidative stress, tissue damage, and blood homeostasis	The amount of selected carbonyls increased as the resistance reduced. Perturbation of the antioxidant and phase II enzymes, increased ROS levels, enhanced xanthine oxidase and cytochrome P450 monooxygenase activity. Disorganization of alveolar and bronchial epithelium in 0.25 Ω group. Alteration in haematocrit and haemoglobin levels, red blood cell and reticulocyte count, as well as lymphocytes and leucocytes profiles with the most marked changed in 0.25Ω group.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Khan et al., 2019, US (227)	To investigate the impact of VP aerosol and waterpipe smoke (WPS) on pulmonary circadian molecular clock disruption in mice	Mice/C57BL6 Male and Female (n=5-7) 14-16 weeks old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG, PG/N, Air VP: eVIC VTC mini Joyetech PG/VG ratio: PG only Nicotine: 0, 25mg/ml Flavour: none VP Settings: 0.15Ω Regime: 70ml/2-3s/puff, 2 puffs/min with 30s interval, 2h/day, for 3 days	Expression levels and abundance of core clock component genes (BMAL1, CLOCK) and clock-controlled output genes (Rev-erbα, Per2, Rev-erbβ, Cry2, Rorα) in mouse lungs	Increased expression and abundance of circadian molecular clock genes (Clock and Per2) and proteins (BMAL1 and PER2) in the lungs of animals exposed to PG/N compared to PG and air-control groups. The expression of Bmal1 gene was upregulated after exposure to PG/N vs. PG, but not air-control.
Wang et al., 2019, US (228)	To examine whether VP aerosols containing PG and nicotine induce sex-dependent lung inflammatory responses and dysregulated	Mice/C57BL6 Male and Female (n=6-9) 15-17 weeks old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG, PG/N, Air VP: eVIC VTC mini Joyetech PG/VG ratio: PG	Inflammatory cell influx BALF levels of pro-inflammatory cytokines (TNFα, IL-1α, IL-1β, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17α, IFNγ, KC, G-CSF, GM-CSF, eotaxin, MCP-1, MIP-1α, MIP-	Exposure to PG/N induced inflammatory cell influx (neutrophils and CD8a+ T lymphocytes), and caused pro-inflammatory mediator release in BALF compared to PG only and air-control groups in a sex-dependent manner. Exposure to PG increased MPO

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
	repair in mice		only Nicotine: 0, 25mg/mL Flavour: none VP Settings: 0.15Ω Regime: 70ml/puff, 3.3s/puff, 2puffs/min, 2h/day, for 3 days	1β, and RANTES) and myeloperoxidase (MPO), a biomarker for neutrophilic infiltration and oxidative stress Myogenic and lipogenic markers, nicotinic acetylcholine receptors and ECM-related proteins in lungs Plasma cotinine	levels in BALF. Both PG and PG/N selectively augmented the lung levels of various homeostasis/repair mediators in a sex-dependent manner, including increased protein abundance and altered gene expression of lipogenic markers (PPARγ, ADRP) and myogenic markers (ACTA2, CTNNB1, fibronectin, α-smooth muscle actin and β-catenin), as well as increased protein abundance of ECM remodeling markers (MMP2), nAChRα3 and nAChRα7.
Szafran et al., 2020, US (229)	To examine lung function and immune responses in a mouse model exposed to nnVP aerosols	Mice/C57BL6 Female (n=11-12) 8-weeks old	Exposure: whole body inhalation exposure using Scireq 3rd-Gen VP generator Groups: PG/VG, PG/VG/F VP: NR PG/VG ratio: 7:3 Nicotine: 0 mg/mL	Lung function measurements Lipid mediator analysis Lung cell immunophenotype Immunoglobulin levels in BALF and serum	Exposure to flavoured nnVP increased lung tidal and minute volumes and tissue damping and elevated IgG1 levels in BALF. Both nnVP groups demonstrated increased percentage of dendritic cells, CD4+ T cells, and CD19+ B cells, increased levels of lipid mediators with anti- and pro-inflammatory properties (2-AG

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			Flavour: vanilla VP Settings: 4.1V, 1.5Ω Regime: 55ml/puff, 3sec/puff every 30sec for 2h/day, 7days/week, for 6 weeks	Gene expression analysis in lungs	and 12-HETE), as well as alteration of gene expression in the lungs.
Taha et al., 2020, Jordan (230)	To investigate the effects of VP aerosol exposure on airway inflammation in an allergen-driven murine model of asthma	Mice/Balb/c Male (n=7-10) 7-9 weeks old All animals were sensitized with ovalbumin (2mg/kg/day via intraperitoneal injection on days 0,7,14)	Exposure: whole body inhalation Groups: PG/VG/N, Air, PG/VG/N/Ova, Air/Ova VP: mini ProTank 2 Kanger PG/VG ratio: 7:3 Nicotine: 18mg/ml Flavour: none VP Settings: 1.8Ω at 5.76W Regime: 5puffs/min, 4sec/116.7ml/puff with 10sec intervals, 1h/day, for 28 days PG/VG/N/Ova: 1% Ova challenge,	Inflammatory cells and inflammatory mediators in BALF and lung tissue	Increased number of inflammatory cells in BALF and reduced levels of transforming growth factor (TGF)-β1 and matrix metalloproteinase (MMP)-2 in lung tissue following VP exposure. Combined VP aerosol and Ova exposure resulted in increased airway recruitment of inflammatory cells (especially neutrophils, eosinophils and lymphocytes), increased level of IL-13 and reduced level of TGF-β1.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			50min nebulization 23-27 days		
Wang et al., 2020, US (231)	To examine whether VP exposure induces lung inflammation and repair responses/extracellular matrix (ECM) remodeling, which Is mediated by nAChRα7	Mice C57BL6 (WT) and nAChR α7 KO Male and Female (n=6-10) 3-4 months old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG, PG/N, Air VP: VTC mini Joyetech PG/VG ratio: PG only Nicotine: 25mg/mL Flavour: none VP Settings: 0.15Ω Regime: 70mL/puff, 3.3s/puff, 3puffs/min, 2h/day, 5days/week for 30 days	Inflammatory cell influx and pro-inflammatory cytokines in BALF Gene expression and/or protein abundance levels of selected MMPs and ECM remodelling markers, inflammatory response markers (p50/p105), myeloid and innate immune response target genes in mouse lungs Serum cotinine levels	WT group exposed to VP with nicotine showed increased inflammatory cellular influx of macrophages and T-lymphocytes including increased proinflammatory cytokines in BALF and increased SARS-Cov-2 Covid-19 ACE2 receptor, whereas nAChRα7 KO mice showed reduced inflammatory responses associated with decreased ACE2 receptor. VP and nnVP aerosol altered MMPs (at both protein and mRNA level) and ECM remodelling proteins in a sex-dependent manner (but not nAChRα7-dependent).
Wirjatmadi et al., 2020, Indonesia (232)	To examine the VP-induced enhancement of free radical within the blood and lung tissue in rats	Rats/Wistar Male (n=5) 2-3 months old	Exposure: whole body inhalation Groups: PG/VG/N, Air VP: NR PG/VG ratio: NR	Malondialdehyde blood levels Malondialdehyde expression within the lung tissue (IHC)	Differentiation of malondialdehyde content within the blood and lung tissue between control and all treatment groups ($p< 0.05$) with a strong and significant relationship between blood and lung

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			Nicotine: 6mg/mL Flavour: NR VP Settings: NR Regime: 5 groups with different time and dose exposure to VP aerosol: 5min/day for 1 week, 2 x 5min/day for 1 week, 5min/day for 2 weeks, 2 x 5min/day for 2 weeks, 5min/day for 3 weeks		malondialdehyde levels (r=.948, p<.001).
Lallai et al., 2021, US (233)	To investigate whether VP aerosol exposure alters ACE2 and nAChR expression in mice	Mice/C57BL 6 Adult male and female	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N, Air VP: NR PG/VG ratio: 1:1 Nicotine: 7.5mg/mL Flavour: none VP Settings: NR Regime: 12 puffs with 5min intervals, 1h/day, for 5 days	ACE2 mRNA and protein expression in lungs and blood The nAChR subunits in lung tissue Blood cotinine levels	Increased ACE2 mRNA and protein in lungs of male mice exposed to nicotine vapour. Downregulated α5 nAChR subunits in lung tissue of males and females following nicotine and vehicle exposure.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Naidu et al., 2021, US (234)	To examine the effects of VP aerosol exposure on ACE2 expression, lung inflammation and lung function in mice	Mice/Balb/c Male and Female (n=5) 7-8 weeks old	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N, Air VP: KangerTech PG/VG ratio: NR Nicotine: 0, 18mg/mL Flavour: NR VP Settings: NR Regime: 55mL/3s/puff with 30s intervals for 30min twice per day, for 21 days	Basal inspiratory capacity and airway responsiveness to methacholine Total cell count, cell differentials, cytokine levels (MCP-1, IL-1B, IL-6, and KC) in BALF ACE-2 expression and localization in lung tissues Lung histology to assess structural integrity and tissue inflammation	VP-exposed animals had increased peribronchiolar inflammation and influx of immune cells into the airways Increased BALF levels of monocyte chemoattractant protein-1, interleukin 1β, and KC in a nicotine-dependent manner in both sexes (IL-6 also increased, but independent of nicotine exposure). The reduction in basal inspiratory capacity following VP exposure, independent of sex or nicotine. Increased airway hyper-responsiveness in both sexes, with nicotine-dependent effect in females, but not males. Lung ACE-2 expression was increased in a nicotine-dependent manner in males but not in females.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Khosravi et al., 2018, US (235)	To investigate the bronchomotor response to VP aerosol inhalation challenge in guinea pigs and the mechanisms involved in regulating these responses	Hartley guinea pigs Male (n=5-9)	Exposure: VP aerosol delivered into the lung via the tracheal cannula under anaesthesia Groups: PG/VG/N, Air VP: Subtank Mini, KangerTech PG/VG ratio: NR Nicotine: 12mg/ml Flavour: NR VP Settings: 5V, 0.5Ω, 50W Regime: a single puff, 6ml/2s/puff (diluted with air in 1:1 ratio), twice with 20min interval	Electrophysiological recording of bronchopulmonary C-fiber activity and measurements of lung mechanics	A single puff of VP aerosol triggered an acute bronchoconstriction that sustained for >2 min. The VP evoked increase in airway resistance was almost completely abolished by a pre-treatment with atropine or lidocaine, suggesting that bronchoconstriction was mediated through the cholinergic pathways. Electrophysiological recording confirmed a stimulatory effect of VP aerosol on vagal bronchopulmonary C-fibers. These effects were primarily driven by nicotine in VP aerosol. A pretreatment with nAChR antagonists completely prevented the VP-induced airway constriction.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Cancer					
Studies comparing effects of VP and air exposure					
Tang et al., 2019, US (236)	To examine tumorigenicity of VP aerosol in mice	Mice/FVB/N Male (n=40 for PG/VG/N, n=18 for PG/VG, n=18 for Air) 6-8 weeks old	Exposure: whole body inhalation using 3-port e-Aerosol Groups: PG/VG, PG/VG/N, Air VP: NR PG/VG ratio: 1:1 Nicotine: 0ng/mL (n=18) or 36mg/mL (n=40) Flavour: none VP Settings: 4V, 1.9A Regime: 4h/day, 5days/week, for 54 weeks	Tumour formation in the lungs, heart, liver, kidneys, intestine, pancreas, brain, spleen, and bladder Proliferation markers MCM-2 and PCNA and the basal cell marker KRT5 in bladder tissue	The tumour-like growth in the skin, abdominal cavity, intestines, and lungs in VP and nnVP groups. Exposure to VP with nicotine induced lung adenocarcinomas (9 of 40 mice, 22.5%, 1 mouse had multiple tumours, all other 1) and bladder urothelial hyperplasia (23 of 40 mice, 57.5%). 1 out of 18 control mice had a single lung adenocarcinoma.
Huynh et al., 2020, US (237)	To investigate the effects of VP exposure on lung colonization of circulating breast cancer cells in mice	Mice/NOD-SCID-Gamma Female 4 weeks old Human breast	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG/N, Air VP: NR	Gross and IVIS examination of lung colonization of breast cancer cells Immunohistological analysis of lung	VP exposure accelerated lung colonization of breast cancer cells. VP exposure almost doubled the level of tumour cells colonised in the lungs (p=.0036) as demonstrated by GFP stain. In

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
		cancer MDA-MB 231 LM2 cells were introduced by tail vein injection	PG/VG ratio: 1:1 Nicotine: 24mg/ml Flavour: none VP Settings: NR Regime: 2h/days, 5days/week, for 4 weeks	colonization of breast cancer cells, quantification of tumour area (GFP positive cells), proliferation (Ki67 positive cells), and apoptotic rate (cleaved Caspase-3 positive cells) Urine cotinine	addition, tumour cell apoptosis was decreased (p<.001, caspase-3 stain) in VP-exposed animals, while the proliferative index was not altered (p=.7953, Ki67 stain).
Pham et al., 2020, US (238)	To elucidate the effects of VP exposure on breast cancer development and lung metastasis along with associated underlying mechanisms	Mice/Balb/c Female (n=8) 5-7 weeks old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG/N, Air VP: NR PG/VG ratio: 1:1 Nicotine: 24mg/ml Flavour: NR VP Settings: NR Regime: 70ml/puff, 1puff/min, 2h/day, 5days/week, for 6 weeks	The number of metastatic tumour nodules in lungs Histological and immunohistochemical analysis to measure primary tumour areas, tumour cell proliferation (Ki-67 stain) and tumour cell apoptosis (cleaved caspas-3 stain) Flow cytometry (monocyte surface markers, CCR1, CCR5, CX3CR1)	VP exposure enhanced breast cancer cell growth in MFP primary tumour and metastatic lung colonisation: VP exposed mice exhibited faster primary tumour growth (2.27-week doubling time vs. 1.24-week in control) and increased tumour area (69.24% vs. 35.46% in control), 100% development of primary tumour or lung metastasis (6/6 vs. 2/6 in control), accompanied by reduced breast cell apoptosis (by 14.98% in primary tumour and by 27.8% in metastasis) and increased proliferation index (by 15.74% in

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			Breast cancer cells were injected orthotopically after 1 week of exposure into the mammary fat pad (MFP)	Urine cotinine	primary tumour and by 20.71% in metastasis). VP exposure promoted an increase in circulating monocytes and infiltration of tumour-associated macrophages in the primary and metastatic tumour microenvironment.
CNS					
Studies comparing effects of VP, TC and air exposure					
Heldt et al., 2020, US (239)	To establish and validate a clinically relevant model of TC and VP use in mice and to characterize the impact on blood-brain barrier (BBB) function	Mice/C57BL6 Male (n=3-10 for molecular studies, n=8-20 for behavioural) 8 weeks old	Exposure: whole body inhalation using Teague system (TE-2) Groups: PG/VG/F, PG/VG/N/F, TC, Air VP: EVOD Mega KangerTech PG/VG ratio: NR Nicotine: 0, 18mg/mL Flavour: tobacco VP Settings: 1.8Ω Regime: 35ml/4s/puff every 30s, 2h/day,	Transcriptional profile within cerebral microvessels, expression of vascular and inflammatory markers, BBB permeability, and leukocyte-endothelial cell interaction Microglial activation and several measures of affective state and cognitive function	Expression of genes with critical roles in BBB function (tight junction-, transport-, and immune-related genes) were up- and down-regulated following exposure to nnVP (2163 and 2281 genes, respectively), VP (311 and 863 genes) and TC (529 and 384 genes). All exposures reduced protein expression of Occludin and Glut1 at the tight junction and increased adhesion of peripheral leukocytes to brain endothelium with a greater magnitude in the absence of nicotine.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			5d/week, for 8 weeks TC: 1R6F, 35ml/puff, 2s/puff, 2 cig with 28s interval (matched serum cotinine to VP)		Only animals exposed to nnVP had increased paracellular permeability and impaired novel object recognition performance, while animals exposed to VP had increased microglial arborization within the striatum.
Ponzoni et al., 2020, Italy (240)	To investigate behavioural and neurochemical effects of withdrawal for up to 90 days after 7 weeks of VP or TC exposure	Mice/Balb/c Male (n=10) 3 months old	Exposure: whole body inhalation Groups: PG/VG/N/F, TC, Air VP: NR PG/VG ratio: 55:35 Nicotine: 5.6mg Flavour: blended mix with vanilline VP Settings: NR Regime: 8ml/puff, 25puffs/min, 3x30min per day (16.8mg of nicotine per day), for 7 weeks TC: 3x 7cig/day (0.8mg/cig,	Behavioural testing 1, 15, 30, 60 and 90 days after the last exposure to assess recognition and spatial memory (virtual and spatial object recognition tests), anxiety (elevated plus maze test) and compulsive-like behaviour (marble burying test), attention-related (virtual object recognition task) and depression-like behaviours (tail suspension and sucrose preference tests) Nicotine and cotinine	The withdrawal (WDW) of VP and TC induced early behavioural alterations, such as impaired attention accompanied by a spatial memory deficit (appeared as early as 15 days after WDW and persisted for 90 days in both groups), increased anxiety (1-30 days post WDW in VP, 1-60 days in TC) and compulsive-like behaviour (60 and 90 days post WDW in both groups), depression-like behaviour and anhedonia (30-90 days post WDW in VP, 60-90 days in TC). Notably, the levels of nicotine and cotinine in the brain were similar between groups. The WDW-induced changes in

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			16.8mg/day of nicotine), for 7 weeks	levels in brain and urine Neurochemical investigations: AMPA and NMDA receptor subunits and PSD95 protein levels, corticotropin-releasing factor (Crf) and Crf receptor 1 (CrfR1) gene expression in the hippocampus	the hippocampal region in both groups: AMPA receptor subunit (GluA2/3 and GluA1) and PSD95 protein levels initially remained unchanged and decreased after 60–90 days, whereas Crf/CrfR1 mRNA expression levels initially increased and then decreased after 60 days (except for initial CrfR1 in TC group that remained unchanged). These late reductions paralleled the development of depression-like behaviours.
Prasedya at al., 2020, Indonesia (241)	To evaluate the in vivo effects of short-term VP exposure on brain inflammatory responses associated with cognitive spatial and memory functions and compare them with the effects of TC smoke	Mice/Balb/c Male (n=6) 3 months old	Exposure: whole body inhalation Groups: PG/VG/N/F, TC, Air VP: 510-T Joyetech PG/VG ratio: 3:7 Nicotine: 18mg/mL Flavour: grape VP Settings: NR Regime: 50ml/3s/puff with 1min intervals, 150puffs/day, for 14	Body weight Learning and memory functions H&E staining of brain tissues to assess histopathological injuries Immunohistochemical analysis of brain tissues (TNF-α)	Reduced body weight gain following exposure to TC smoke and VP aerosol. Both TC and VP exposure caused reduced cognitive spatial learning abilities (delayed time in finding food reward), but VP group also showed reduced memory functions (finding the reward the next day) compared to TC and control groups. Inflammatory characteristics such

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			days TC: 6 cig/day, for 14 days		as necrotic cells and cytoplasmic vacuolization in the cerebral cortex of mice brain in both VP and TC-exposed groups High expression of inflammatory marker TNF-α in the brain tissues of both groups
Carboni et al., 2021, Italy (242)	To examine the alterations in key neurotransmissions after 60 days of withdrawal from 7-week intermittent exposure to TC smoke, VP or nnVP aerosols	Mice/Balb/c Male (n=4-32)	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N/F, TC, Air VP: NR PG/VG ratio: 55:45 Nicotine: 0mg or 5.6mg/30min session Flavour: blended mix with vanilline VP Settings: NR Regime: 8ml/puff, 25puffs/min, 3 x 30min/day (16.8 mg of nicotine/day), for 7 weeks TC: 3 x 7cig/day	Assessment of behaviour at 60 days of withdrawal: depressive-like behaviours (the tail suspension and sucrose preference tests), anxiety- or obsessive–compulsive-related behaviours (the marble burying test), cognitive impairments (spatial object recognition test) Gene expression of the neuropeptide systems involved in the neuroadaptations, including Corticotropin-releasing factor (Crf), Dynorphin, Nociceptin	After 60-day withdrawal from nicotine exposure, TC and nicotine-containing VP groups demonstrated cognitive impairments, increased depressive and anxiety/obsessive–compulsive-like behaviours compared to controls. No difference in nnVP group except for the marble burying test, which probes anxiety-like/compulsive behaviour. Increased Crf and Crf receptor 1 (Crf1) mRNA levels specifically after TC withdrawal in the CPu. The nociceptin precursor prepronociceptin levels were

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			(matched nicotine dose), same regime	system, Enkephalin, Orexin/Hypocretin, and Bdnf systems, in hippocampus (Hip) and caudate-putamen (CPu) after 60 days of withdrawal from exposures	reduced by TC (80%) and nicotine VP (50%) withdrawal in the CPu. The delta opioid receptor showed a reduction in Hip driven by VP exposure independent of nicotine, while the Dop levels doubled in the CPu of mice exposed to nicotine containing VP only. Withdrawal after exposure to nicotine containing VP induced a 35% Bdnf mRNA decrease in Hip, whereas Bdnf was augmented by 118% by TC withdrawal in CPu. No alterations were induced by 60-day withdrawal in dynorphin-ergic or orexin/hypocretin-ergic systems in Hip and CPu.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Studies comparing effects of VP and air exposure					
Alasmari et al., 2019, US (243)	To examine the effects of chronic inhalation of VP aerosol containing nicotine on neurotransmitters in the mesocorticolimbic brain regions in mice	Mice/C57BL 6 Male (n=10) 6-8 weeks old	Exposure: nose-only inhalation using Scireq Inexpose system Groups: PG/VG/N, Air VP: FastTech PG/VG ratio: 1:1 Nicotine: 24mg/mL Flavour: none VP Settings: 2.4 Ω, 280mAh Regime: 4s puff every 20s for 1 h/day, for 5 days/week, 6 months	Dopamine, serotonin, GABA, glutamate and glutamine concentration in frontal cortex (FC) and striatum (STR)	Chronic VP exposure decreased dopamine and increased both glutamine and glutamate in STR, while also increased glutamine and decreased GABA in FC.
Alasmari et al., 2021, US (244)	To investigate the effects of chronic exposure to nicotine-containing VP aerosol on the expression of nicotinic receptor and astroglial	Mice/C57BL 6 Female (n=5) 6–8 weeks old,	Exposure: nose-only inhalation using Scireq Inexpose system Groups: PG/VG, PG/VG/N, Air VP: FastTech PG/VG ratio: 1:1	Protein levels of α7 nAChR, α4/β2 nAChR and GLT-1 isoforms Gene expression and protein levels of astroglial glutamate transporters, including glutamate transporter-1	VP exposure, but not nnVP, altered the expression of nicotinic receptors and astroglial glutamate transporters in specific mesocorticolimbic brain regions. This included increased α4/β2 nAChR in all brain regions, and increased α7 nAChR expression in the FC and STR. The total

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
	glutamate transporters, ARC and BDNF in mesocorticolimbic brain areas of chronically exposed mice		Nicotine: 0, 24mg/mL Flavour: none VP Settings: 1.8Ω tank and 3.4V/280mAh battery Regime: 4s puff every 20s for 1 h/day, 5 days/week for 3 months	(GLT-1) and cystine/glutamate antiporter (xCT), in the frontal cortex (FC), striatum (STR) and hippocampus (HIP) Activity-regulated cytoskeleton-associated protein (ARC) and brain-derived neurotrophic factor (BDNF) in STR Cotinine levels in FC and STR	GLT-1 relative mRNA and protein expression were decreased in STR only, while GLT-1 isoforms (GLT-1a and GLT-1b) were downregulated in the STR in VP group. There was a marked increase in BDNF protein expression in the VP group in the STR compared to both nnVP and control groups. Also, high cotinine concentration was detected in the FC and STR in VP group.
Cardenia et al., 2018, Italy (245)	To evaluate the impact of VP aerosol on rat brain lipid profile	Rats/Sprague Dawley Male (n=10) 8 weeks old	Exposure: whole body inhalation Groups: PG/VG/N/F, Air VP: NR PG/VG ratio: NR Nicotine: 18mg/ml Flavour: red fruit VP Settings: 5.5V, 2Ω, 15W Regime: 11 x 2 puffs (6s puff, 5s	Total lipid content, fatty acid and sterol composition, as well as oxysterol content in brain Principal component analysis (PCA)	Following 4-week exposure to VP aerosol, the oxysterol formation was reduced ($p<.05$), except for triol and 5a, 6a-epoxycholesterol, while no effect was found after 8 weeks. 8-week VP exposure led to increase of saturated fatty acids ($p<.05$), including palmic acid, and decrease of polyunsaturated fatty acids ($p<.05$), especially arachidonic and

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			interval) followed by 20min recovery, 5day/week, for 4 weeks and 8 weeks		docosahexaenoic acids. Both atherogenic (AI) and thrombogenic (TI) indices were found to be increased (p<.05). Also, cholesterol content was reduced (p<.05), while 7-dehydrocholesterol (7-DHC) was increased (p<.05) after 8-week treatment. PCA separated all VP from control groups, evidencing that oxysterols (except triol and 24(S)-hydroxycholesterol) were inversely correlated to 7-DHC and TI.
Sifat et al., 2019, US (246)	The effects of VP aerosol exposure containing nicotine on ischemic brain glucose utilization	Mice/C57BL 6 Male (n=3-8) 6 months old	Exposure: whole body inhalation Groups: PG/VG/N, Air VP: NR PG/VG ratio: NR Nicotine: 24mg/ml Flavour: NR VP Settings: NR Regime: 35ml/4s/puff every	Brain slices viability Measurement of brain glucose uptake Protein levels of glucose transporters (GLUT1, GLUT3)	7-day VP exposure resulted in decreased brain glucose uptake under normoxic and ischemic conditions along with downregulation of GLUT1 and GLUT3 expressions

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			60s, 16puffs/session, 6times/day, 1 cartridge/day, 6-8h/day, 7days/week, for 1 week		
Alhaddad et al., 2020, US (247)	To evaluate the effect of three months' continuous exposure to nicotine-containing VP aerosol (JUUL pods) on the expression of glutamate receptors and transporters in drug reward brain regions	Mice/C57BL6 Female (n=5-6) 6-8 months old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG/N/F, Air VP: JUUL pod PG/VG ratio: NR Nicotine: 5% (59 mg/mL) Flavour: mint or mango VP Settings: NR Regime: 4s/puff with 16s interval, 20min/day, 5days/week, 3 months	Protein expression of metabotropic glutamate receptors (mGluR1 and mGluR5), glutamate transporter 1 (GLT-1), cystine/glutamate antiporter (xCT), phospho-postsynaptic density protein p-PSD95 and PSD95 measured in the nucleus accumbens core (NAc-core), nucleus accumbens shell (NAc-shell) and hippocampus (HIP)	3-month JUUL exposure induced upregulation of mGluR1 ($F_{2,14}=7.35$, $p=.006$) and phosphorylated ($F_{2,14}=5.31$, $p=.019$) and total PSD95 ($F_{2,14}=9.07$, $p=.003$) expression, and downregulation of mGluR5 ($F_{2,14}=9.63$, $p=.002$) and GLT-1 ($F_{2,14}=10.18$, $p=.0019$) in the NAc-shell. In addition, exposure to JUUL was associated with upregulation of mGluR5 ($F_{2,14}=7.02$, $p=.007$) and GLT-1 ($F_{2,12}=20.41$, $p=.0001$) expression in the HIP. Difference between mint and mango group was only found in GLT-1 expression in HIP ($p<.05$) No significant change in xCT

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
					expression.
Ni et al., 2020, US (248)	To examine the effects of VP aerosol exposure on neuron activation in trigeminal ganglion and brainstem nuclei	Mice/ C57BL6 ChAT(BAC) -eGFP transgenic mice Male and Female 3-6 months old	Exposure: whole body inhalation Groups: PG/VG/F, Air VP: Evod pro V2 KangerTech PG/VG ratio: 1:1 Nicotine: 18mg/mL Flavour: vanilla VP Settings: 30W,0.5Ω Regime: 60mL of aerosol injected at 0, 8, and 21 min during 30-min session	Quantitative analysis of activated-trigeminal nociceptive neurons and brainstem neurons	VP aerosol exposure increased numbers of activated trigeminal nociceptive neurons and brainstem neurons in the spinal trigeminal nucleus, paratrigeminal nucleus, and nucleus tractus solitaries.
Chen et al., 2021, Australia (249)	To investigate whether VP aerosol inhalation interacts with high-fat diet (HFD) to affect short-term memory and neural integrity	Mice/Balb/c Male (n=10) 7 weeks old The population was divided into normal chow (14% fat) or HFD	Exposure: whole body inhalation Groups: PG/VG/F, PG/VG/N/F, Air VP: NEBOX Kanger Tech PG/VG ratio: 1:1 Nicotine: 0, 18mg/mL	Memory behaviour Neural cell integrity markers Brain cell levels Synaptic protein markers	VP exposure regardless of nicotine impaired short-term memory function in chow-fed mice. Exposure to VP increased systemic cytokines (serum IL-1β and TNFα, p<.05 nnVP only), increased brain p-Tau (p=.084 nnVP and p=.054 nicotine VP)

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
		(43% fat), for 10 weeks prior exposure to induce obesity	Flavour: tobacco VP Settings: NR Regime: 4 x 5s puff at 30W with 20s interval, 2 x 15min/day, equivalent nicotine exposure to 2cig twice daily, for 6 weeks	Brain insulin pathway markers Inflammation, apoptosis, and oxidative stress responses	and glial fibrillary acidic protein (GFAP, p<.01 nnVP only), and decreased the number of neurons (p<.05 nicotine VP only) and postsynaptic density protein (PSD)-95 levels (p<.01 both) in chow-fed mice. Also, decreased astrocyte marker GFAP (p<.05 nicotine VP and p<.01 nnVP), increased microglial marker Iba-1 (p<.05 nnVP only) and increased glycogen synthase kinase (p<.01 both) levels in HFD-fed mice. Increased hippocampal apoptosis was also differentially observed in chow and HFD mice following exposure to VP.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Digestive and reproductive					
Studies comparing effects of VP and air exposure					
Espinoza-Derout et al., 2019, US (250)	To examine the harmful effects of VP on the liver with a special emphasis on DNA damage and mitochondrial dysfunction	Mice/C57BL 6 ApoE-/- Male (n=5) 8 weeks old Western diet with 45% fat	Exposure: whole body inhalation Groups: PG/VG/F, PG/VG/N/F, Saline VP: bluCig PLUS PG/VG ratio: NR Nicotine: 0, 24mg/mL Flavour: tobacco VP Settings: NR Regime: 4s/puff, 8puffs with 25s intervals every 30min, repeated 24 times daily for 12h, for 12 weeks	Markers of hepatic DNA damage (apurinic/apyrimidinic, AP, sites, NAD+ and NADH liver content, NAD+/NADH ratio), oxidative stress (Malondialdehyde, MDA), hepatic mitochondrial dysfunction (PTEN-induced kinase 1, PINK1), and activation of Poly (ADP-ribose) polymerases (PARP1 and Sirtuin-1, SIRT1)	Mice exposed to VP with nicotine had increased AP site lesions, decreased NAD+/NADH ratio with induction of hepatic NADH levels and depletion of NAD+ levels, as well as increased levels of oxidative stress (MDA), all compared to control ($p<.05$) and nnVP ($p<.05$) groups, without the changes between nnVP and control groups. Additionally, VP with nicotine produced an increase in PARP1 activity associated with reduced protein levels of SIRT1, and increased levels of PINK1 protein and mitochondrial DNA mutations associated with mitochondrial ultrastructural changes (increased vacuolization and a reduction in cellular organelles). $P<.05$, VP with nicotine vs. control.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Hasan et al., 2019, US (251 249)	To examine the harmful effects of VP exposure on the liver	Mice/ApoE−/− Male (n=6) 8 weeks old Western diet containing 0.21% cholesterol, calories from fat (21%), carbohydrates (50%) and protein (20%)	Exposure: whole body inhalation Groups: PG/VG/F, PG/VG/N/F, Saline VP: NR PG/VG ratio: Nicotine: 0, 24mg/mL Flavour: tobacco VP Settings: NR Regime: 4s/puff, 8puffs with 25s intervals every 30min, repeated 24 times daily for 12h, for 12 weeks	Assessment of hepatic oxidative stress (4-hydroxytrans-2-nonenal, 4-HNE), lipid accumulation (hepatic triglyceride levels, histological evaluation including H&E, light microscopy and TEM), hepatocyte apoptosis and caspase activation (positive nuclei per total nuclei, active caspase 9 and caspase 3), fibrosis (collagen content, TGF-α and TGF-β), and AMP-activated protein kinase (AMPK)-mediated pathways (protein expression of total and phospho-AMPK, p-AMPK, total and phospho-ACC, p-ACC, sterol regulatory element binding protein lc, SREBP1c, and fatty acid synthase, FAS peroxisome	ApoE-/- mice on a WD exposed to VP with nicotine had increased hepatic lipid accumulation, higher triglyceride levels ($p<.05$), greater oxidative stress (4-HNE) and increased hepatocyte apoptosis (by 2.0-fold, plus increased caspase 9 and 3, $p<.05$), independent of AMP-activated protein kinase (AMPK) signalling, compared to saline treated animals. No changes in the hepatic expression of SREBP1c, FAS, phospho-ACC, PPAR δ, INSIG1, collagen content, TGF-α and TGF-β. In addition, 433 genes were differentially expressed ($p<.05$) with genes associated with lipid metabolism, cholesterol biosynthesis, and circadian rhythm being most significantly altered following exposure to VP with nicotine. No changes in collagen deposition, little or no hepatic lipid

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
				proliferatoractivated receptor δ, PPAR δ, and insulin-induced gene 1, INSIG1) Gene expression analysis and RNA sequencing analysis Plasma cotinine	accumulation were reported in nnVP group.
Vivarelli et al., 2019, Italy (252)	To investigate the effects of nnVP aerosol generated from a low-voltage device on rat testicular functions	Rats/ Sprague Dawley Male (n=6-7) 8 weeks old	Exposure: whole body inhalation using EcigAero system Groups: PG/VG/F, Air VP: NR PG/VG ratio: 1:1 Nicotine: 0 mg/mL Flavour: 10% red fruit VP Settings: 3000mAh/3.7V, 1.5Ω Regime: 11 x 2 puffs (6s puff, 5s interval), followed by	Body weight, relative testis weight Testicular androgenic enzymes activities: 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD) Testicular marker enzymes: sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH), glucose-6-phosphate	VP exposed rats had a lower relative testis weight (≈14% loss, $p<.05$) and a raise of LDH as tissue damage marker, along with an impairment of steroidogenesis enzymes (decreased 3β-HSD, 17β-HSD, SDH and G6PDH). The pro-oxidative environment was confirmed by increased levels of ROS, PC, MDA and LOODH ($p<.01$), as well as by the disruptive effect of VP on the antioxidant and detoxifying enzymatic systems (decreased CAT, NQO1, SOD, GST, UDP-GT, $p<.01$, while increased XO, $p<.01$).

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			20min of recovery, 3h/day, for 28 days	dehydrogenase (G6PDH) Testicular oxidative stress markers: reactive oxygen species (ROS), protein carbonyl groups (PC), malondialdehyde (MDA), lipid hydroperoxides (LOOHs) Antioxidant and detoxifying enzymes: catalase (CAT), NAD(P)H:quinone reductase (NQO1), superoxide dismutase (SOD), oxidized glutathione reductase activity (GSSG-red), glutathione peroxidase (GSH-Px), glutathione S-transferase, (GST), UDP-glucuronosyl transferase (UDP-GT); and xanthine oxidase (XO)	Additionally, a higher rate of DNA unwinding was observed in white blood cell line ($p<.05$) and boosted CYP-linked activity (CYP1A1, CYP1A1/2, CYP2B1/2, CYP2E1, $p<.01$) as well as LOX-linked activity ($p<.01$), a tumour promotion marker.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
				Analysis of DNA strand breaks in white blood cells Cytochrome P450 (CYP) -linked activities (CYP1A1, CYP1A1/2, CYP2B1/2, CYP2E1) and Lipoxygenase (LOX)	
Li et al., 2020, Australia (253)	To understand the impact of intrauterine VP exposure on liver metabolic markers in the male offspring	Mice/Balb/c Female breeders (n=8), 7 weeks old Males offspring were weaned at postnatal day 20	Exposure: whole body inhalation using Kanger Tech Groups: PG/VG/F, PG/VG/N/F, Air VP: NEBOX KangerTech PG/VG ratio: 1:1 Nicotine: 0, 18mg/mL Flavour: tobacco VP Settings: 0.5Ω,30W Regime: 2 times per day, for 6 weeks before mating and	Body and liver weight, liver triglyceride (TG) levels Plasma activity of alanine transaminase (ALT), insulin, nonesterified free fatty acid (NEFA) and triglyceride (TG) levels An intraperitoneal glucose tolerance test (IPGTT) in male offspring at 12 weeks HOMA-IR index, an	Exposure to nnVP promoted metabolic changes and liver damage in both the dams and their offspring. Exposure to nnVP in dams promoted insulin resistance and impaired insulin receptor pathway activation (increased Glut4, HOMA-IR, decreased p-Akt), increased hepatic TG and plasma NEFA concentrations, impaired liver mitochondrial health (decreased mtDNA-CN and PGC1α, increased DRP1) and induced hepatocellular damage associated with increased

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			throughout gestation and lactation	indicator of insulin resistance Gene and protein expression in dams and offspring, including glucose metabolic markers (insulin-independent glucose transporters, Glut2, Glut4), markers for glycolysis and gluconeogenesis (phosphofructokinase1, PFK1, and forkhead box protein O1, FOXO1), hepatic protein markers of insulin signaling (p-Akt) and lipid synthesis (FASN), markers of oxidative stress (nitrotyrosine), mitochondrial antioxidants (MnSOD and Gpx1) and inflammation (F4/80, TNF-α, and IL-1β),	oxidative stress along with increased inflammation and macrophage infiltration (increased plasma ALT, nitrotyrosine staining, F4/80+ cell numbers, IL-1β, decreased MnSOD). Higher levels of plasma ALT and hepatic DRP1 and LC3A/B II expression were found in nnVP relative to VP with nicotine group. Similarly, nnVP exposure in offspring led to reduction in the mitochondrial number (increased mtDNA CN, LC3A/B II), increased oxidative stress and inflammation (increased nitrotyrosine staining, TNF-α, and decreased GPx1) associated with increased glucose uptake and altered levels of hepatic glucose metabolic markers (increased IPGTT, Glut2, Glut4, PFK1, FOXO1), with the majority of markers being significantly different from VP with nicotine group.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
				mitochondrial DNA copy number, mtDNA CN, markers of mitophagy (DRP1 and OPA1), autophagy (LC3A/B II), and mitochondrial biogenesis (PGC1α)	Exposure to nicotine-containing VP did not change hepatic markers of glucose and lipid metabolism, inflammation and oxidative stress, but caused insulin resistance in dams (increased Glut4 and HOMA-IR associated with decrease in p-Akt) and induced hepatic steatosis in the adult offspring (increased liver TG and FASN protein level).
Nima et al., 2020, Iraq (254)	To examine oxidative stress induced by vapours of E-hookah on mice liver tissues	Albino mice Male (n=10) 2-3 months	Exposure: whole body inhalation Groups: PG/VG/N/F, Air VP: SIDIA8 Vape PG/VG ratio: NR Nicotine: 3% Flavour: fruits VP Settings: 45W Regime: 15s/puff with 15s interval, 20min with 5min rest, 1h per day for 30 days	Total body weight, relative weight of liver, kidney and spleen Liver tissue content of malondialdehyde, total protein carbonyl and nitric oxide	1 month exposure to E-hookah resulted in increased relative weight of liver and spleen (p=.0054 and p=.0005), but not kidney. No difference in body weight. Higher levels of oxidative stress markers, such as malondialdehyde (p=.0004), total protein carbonyl (p=.0001) and nitric oxide (p=.0025), were found in E-hookah-exposed animals.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Sharma et al., 2021, US (255)	To assess the effects of VP use on the gut barrier	Mice/C57BL6 Male or Female (n=4) 6-8 weeks old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG, PG/VG/N, Air VP: KangerSubtank PG/VG ratio: 7:3 Nicotine: 0, 6mg/mL Flavour: none VP Settings: 0.15Ω Regime: 4s every 20s for 1h/day, 5days/week, for 1week or 3 months	Histologic and transcriptome analyses of colon: markers of gut epithelial tight junctions (TJs), e.g., occludin (OCLN), zonula occludens (ZO)-1 (TJP1), Claudin-1 (CLDN1), Claudin-2 (CLDN2), pro-inflammatory cytokines MCP1 or IL-8	Submucosal inflammatory infiltrates were present in the colon of all VP-exposed mice following acute and chronic exposures with a higher extent in nicotine free group at 3 months. Also, infrequent patches of epithelial erosions were observed after 1-week exposure to nicotine VP only. Animals chronically exposed to nicotine-free aerosol had reduced expression of genes related to epithelial TJs (OCLN, TJP1 and CLDN2) compared with nicotine VP and control groups. No difference in the transcript levels of TJ markers and pro-inflammatory cytokines between groups. Chronic exposure to nicotine-free VP was associated with an upregulation of 120 genes (including MCP1, IL-8, and TNF-α) and downregulation of 75

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					genes with most of the differences being abolished in nicotine VP group. Exposure of murine enteroid-derived monolayers (EDMs) to nicotine-free VP aerosols showed a direct disruptive effect on the epithelial barrier.
Multiple					
Studies comparing effects of VP and air exposure					
Crotty Alexander et al., 2018, US (256)	To examine whether VP exposure diminish airway barrier function, leading to inflammatory protein release into circulation, creating a systemic inflammatory state, ultimately leading to distant organ injury and dysfunction	Mice/ C57BL6 and CD1 Female (n=6) 6-8 week old	Exposure: nose-only inhalation using Scireq Inexpose system Groups: PG/VG/N, Air VP: FastTech PG/VG ratio: 1:1 Nicotine: 24mg/mL Flavour: none VP Settings: 3.4V battery and 1.8Ω tank Regime: 4s puff every 20s for 1h/day, 5days/week,	Inflammatory proteins in serum, including fibr matrix metalloprotease-3 (MMP-3), WISP-1, WNT1-inducible signaling pathway, Angiopoietin. Measurements of renal function (glomerular filtration rate), cardiac function (HR, BP) Analysis of renal, cardiac and liver fibrosis, fibrosis markers in renal and cardiac	VP vapour inhalation increased the levels of circulating pro-inflammatory (LIF, EGF, Angiopoietin) and profibrotic proteins (greater effect in C57BL6), induced renal dysfunction and fibrosis in kidney, heart and liver, altered cardiovascular function with decreased heart rate and elevated blood pressure.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			3months (C57BL6) and 6 months (CD-1)	parenchyma	
Lechasseur et al., 2017, Canada (257)	To investigate pulmonary and systemic expression of circadian molecular clock genes following exposure to VP generated vapours of PG and VG in mice	Mouse/Balb/c Female (n=5) 6-8 weeks old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG, VG, PG/VG, Air VP: NR PG/VG ratio: 7:3 or PG only, VG only Nicotine: 0 mg/mL Flavour: none VP Settings: NR Regime: 3 x 80-mL puff/min, 2h/day, 5day/week, for 8 weeks	Expression of circadian molecular clock genes in the lung, brain, liver, kidney and skeletal muscle	Exposure to PG and VG, in combination or separately, modulated the expression of circadian molecular clock genes in lungs, brain, liver, kidney, and skeletal muscle.
H. W. Lee et al., 2018, US (258)	To measured DNA damage induced by nitrosamines in different organs of VP exposed mice	Mice/FVB N Male (n=10)	Exposure: whole body inhalation exposure using e~Aerosols Groups: PG/VG/N, Air VP: NJOY	γ-OH-PdG and O6-medG adduct detection in lung, bladder, and heart tissues DNA repairs proteins detection (XPC and OGG1/2) in lung tissue	Increased DNA adducts in bladder, heart and lung, reduced DNA repair activity, and DNA repair enzyme levels in lung following VP exposure.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			PG/VG ratio: 1:1 Nicotine: 10mg/mL Flavour: none VP Settings: 4.2V Regime: 35mL x 4s puff at 30s intervals 3h/d, 5d/w for 12 weeks		
Chen et al., 2019, China (259)	To examine the effect of VP aerosol exposure on exercise performance and health-related profiles in mice	Mice/ICR Female (n=6) 8 weeks old	Exposure: whole body inhalation exposure Groups: PG/VG, PG/VG/F, PG/VG/N/F, Air VP: Joyetech TCR PG/VG ratio: NR, but adjusted with 4ml VG in PG/VG/N/F only Nicotine: 0, 0.5 or 5mg/mL Flavour: vanilla VP Settings: 4.7V, 0.5Ω, 50W Regime: 30min/day, for 14 days	Exercise performance (forelimb grip strength and weight-loaded swimming test) Tissue glycogen determination, visceral organ weight and tissue histology (liver, kidney, heart, lung, muscle, ovarian fat pad, brown adipose tissue) Clinical biochemical profiles: aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, creatinine, blood	VP exposure led to dose-dependent decrease in the forelimb grip strength (36.21% lower in VP-5 group vs. VP) and swimming time (p<.0001 and p=.0873 in the trend analysis, respectively). The VP-treated groups also showed a dose-dependent decrease in liver (40.78% lower in VP-5 group vs. VP) and muscle (25.60% lower in VP-5 vs. VP) glycogen storage (p=.0009 and p=.0003, trend analysis). No significant difference between air-control, VP, nnVP and VP-0.5 groups.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
				urea nitrogen (BUN), total protein (TP), uric acid (UA), total cholesterol (TC), triacylglycerols (TG) and lactic dehydrogenase (LDH)	No negative effect on levels of biochemical indices. The body weight of the mice did not differ among the groups. No adverse effect or gross abnormalities on the morphology of the major organs.
Kuntic et al., 2020, Germany (82)	To investigate the potential cardiovascular, pulmonary, and cerebrovascular consequences following VP aerosol exposure in smokers and experimental animals	Mice/ C57BL6 and Nox2-/- Male (n=3-38) 9-16 weeks old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG, PG/VG/N, Air VP: eVIC-VTC Mini Joyetech PG/VG ratio: 1:1 Nicotine: 0, 12mg/mL Flavour: none VP Settings: 0.5Ω, 24W Regime: 55ml/3s/puff every 30s, 6x20min, 2h/day, 1,3,or 5 days	Detection of vascular and endothelial function and oxidative stress in cardiac tissue, brain and aorta: systolic and diastolic blood pressure, plasma bilirubin, lipid peroxidation (4-hydroxynonenal, 4-HNE), protein tyrosine nitration (3-nitrotyrosine, 3-NT), ROS formation, inflammation (interleukin-6, IL-6, and CD68), leukocyte-dependent oxidative burst, oxidative stress (2-hydroxyethidium, triphenylphosphonium-linked 2-hydroxyethidium). Brain	VP exposure resulted in detrimental effects on endothelial function, caused oxidative stress in the lung, brain and vessels, induced inflammation, and lipid peroxidation with a higher extent in nicotine free VP group compared to nicotine-containing VP group. These effects of VP exposure were largely absent in mice lacking phagocytic NADPH oxidase (NOX-2) or upon pharmacological ET-1 receptor blockade and FOXO-3 activation by macitentan and bepridil, respectively.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			Some treated with the endothelin ET-1 receptor blocker macitentan (30 mg/kg/d) and the FOXO-3 activator bepridil (20 mg/kg/d)	mRNA expression of NADPH oxidase 1 (Nox1), neuronal nitric oxide synthase (Nos1) and Forkhead-Box-Protein O3 (Foxo3), aortic protein expression of endothelial NO-synthase (eNOS), dihydrofolate reductase (DHFR), endothelin-1 (ET-1), heme oxygenase-1 (HO-1), mitochondrial aldehyde dehydrogenase (ALDH-2), NADPH oxidase subunit NOX-2, neuronal NOS (nNOS), CD68, and protein-acrolein adducts in lung tissue	
Jin et al., 2021, US (260)	To investigate the in vivo cardiopulmonary effects of direct inhalation exposures to PG-VG-derived	Mice/ C57BL6 Male and Female (n=2-12)	Exposure: whole body inhalation using Scireq integrated Cigarette Smoking Robot Groups: PG/VG, Air	Levels of FA and AA in aerosol Biomarkers of exposure and cardiopulmonary injury: urine metabolites (formate, acetate),	Acute PG-VG exposure affected pulmonary reflex (decreased respiratory rate, -50%), endothelium-dependent relaxation, decreased WBC, and increased RBC and haemoglobin.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
	aerosol, and two abundant saturated aldehydes, formaldehyde (FA) and acetaldehyde (AA)		VP: bluPLUS PG/VG ratio: 1:1, 3:7 and 7:3 or either FA or AA without PG/VG Nicotine: 0 mg/mL Flavour: none VP Settings: 3.7V, 8W, 3Ω Regime: 91mL/4sec puff, 2puffs/min for 9min, either 3 cycles in 1 hour (for respiratory parameters) or 20 cycles in 6hours/day, for 4 days	respiratory reflexes, aorta reactivity, blood count and plasma markers (HDL, cholesterol, LDL, total protein, albumin, triglycerides, ALT, AST, LDH, CK and creatinine), blood level of circulating angiogenic cells (CACs) and platelet-leukocyte aggregates (PLAs), endothelial dysfunction	Induced irritant reflex or endothelial dysfunction following exposure to FA, but not AA. PG-VG aerosol exposure increased post-exposure urinary acetate, but not formate.
Systemic					
Studies comparing effects of VP, TC and air exposure					
Orimoloye et al., 2019, US (166)	To assess the association between VP and TC exposures and insulin resistance	Mice/ C57BL6 Male (n=25) 8 weeks old	Exposure: whole body inhalation using e~Aerosols Groups: PG/VG, PG/VG/N, Air VP: NJOY PG/VG ratio: 1:1	Body and organ weight Assessment of insulin resistance (HOMA-IR) and glucose tolerance tests (GTT) Urine levels of nicotine,	Urine nicotine levels and TNC in VP-exposed mice were 4- and 1.8-fold higher than in TC group, respectively, while cotinine and 3HC levels were similar across groups. Body and organ weight, fasting

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			Nicotine: 0, 36mg/mL Flavour: none VP Settings: 4.2V Regime: 35ml/4s/puff with 30s intervals, 3h/day, 7days/week, for 12 weeks TC: 3R4F, 35ml/puff, 2s/puff, 9puffs, 9min/cig, 1cig every 30min = 12cig/6h (matched nicotine exposure), 7 days/week, for 12 weeks	cotinine, 3-hydroxy cotinine (3HC) and total nicotine equivalent (TNC)	blood glucose, insulin, HOMA-IR and GTT levels in TC and VP treated animals were comparable with air-control group.
Wawryk-Gawda et al., 2019, Poland (261 579)	To compare the in vivo effects of VP aerosol and TC smoke exposure on weight gain and glycaemia	Rats/ Wistar Male (n=10)	Exposure: whole body inhalation Groups: PG/VG/N, TC, Air VP: NR PG/VG ratio: PG only with water Nicotine: 12mg/mL Flavour: none	Metabolic parameters (consumed water and food, excreted urine and faeces) Body weight and blood glucose levels at 6 and 8 weeks after the first exposure (6-week exposure followed by 2-	Lower weight gain following 6-week exposure to VP and TC compared to controls (both p=0.01) TC, but not VP, exposed group had higher weight gain at 8 weeks (after 2-week nicotine cessation) compared to controls (p=0.04)

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			VP Settings: 5.5V Regime: 5min/puff with 20min stop, 0.6ml of e-liquid/day, 5days/week, for 6 weeks TC: 10cig/day, 7mg nicotine/day (matched nicotine dose, 210mg in 6 weeks)	week cessation)	Higher blood glucose levels in TC exposed group at 6 and 8 weeks compared to VP (p=0.04, p=0.01) and controls (p=0.01, p=0.03)
Lan et al., 2020, China (262)	To explore the effects of VP on insulin sensibility in ApoE gene knockout mice	Mice/ApoE-/- Male (n=12) 6 weeks old	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N, TC, Air VP: NR PG/VG ratio: NR Nicotine: 0, 12mg/mL Flavour: NR VP Settings: NR Regime: 3x30min/day, for 18 weeks	Body weight Blood lipids: total cholesterol (TCh), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C); and inflammatory indexes (hs-CRP and TNF-α) Insulin tolerance	Exposure to TC and VP, independent of nicotine, reduced body weight gain (p<.05), increased the levels of serum lipids (TCh, TG, LDL-C and HDL-C, p<.05) and inflammatory markers (hs-CRP and TNF-α, p<0.05) with higher levels of TCh, TG and TNF-α observed in TC group vs. VP with or without nicotine (p<.05). Furthermore, insulin tolerance was decreased after exposure to TC (p<.01) and VP with, but not

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			TC: 0.8mg nicotine, same regime		without, nicotine (p<.05).
Studies comparing effects of VP and air exposure					
Ramanathan et al., 2020, US (263)	To assess the effect of VP aerosol on bone marrow hematopoietic stem and progenitor cells (HSPC) number and function using a murine model	Mice/C57BL6 Female (n=4-7) 8 weeks old	Exposure: nose-only inhalation Groups: PG/VG/N/F, Air VP: Vibe PG/VG ratio: 1:1 Nicotine: 15mg/mL Flavour: tobacco VP Settings: 70W Regime: 55ml/2s/puff with 28s intervals, 2h/day, 4days/week, for 2 months Additionally, lethally irradiated recipient mice were transplanted with the whole bone marrow from VP-exposed mice or from VP-exposed mice combined with	Spleen weight Peripheral blood cell count and bone marrow cellularity Bone marrow myeloid progenitor populations including lineage negative, c-Kit positive (LK; Lin−c-Kit+) cells, common myeloid progenitors (CMP; Lin−c-Kit+Sca-1−CD34+CD16/32−), granulocyte-macrophage progenitors (GMP; Lin−c-Kit+Sca-1−CD34+CD16/32+) and megakaryocyte-erythroid progenitors (MEP; Lin−c-Kit+Sca-1−CD34−CD16/32−) Bone marrow	VP-exposed mice had decreased number of bone marrow HSPCs (p=.054 LKS, p=.14 LKS-SLAM) and suppressed myeloid progenitor populations, such as LK and CMP, with a trend towards decreased GMP (p=.08), but not MEP. There was no effect on peripheral blood cell counts, bone marrow cellularity or spleen weight. HSC function was inperturbed in bone marrow transplants following VP exposure (no effect on the peripheral blood cell count post-transplant) While acute LPS treatment did not affect competitive fitness in VP-exposed group, mice transplanted with bone marrow from VP-exposed mice that were challenged with LPS had elevated monocytes in the

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			an inflammatory challenge of a single dose of lipopolysaccharide (LPS) in competitive transplantation experiments	hematopoietic stem (HSC) and progenitor cells (PC), including LKS (Lin−c-Kit+Sca-1+) and LKS-SLAM (LKS-CD48-CD150+) populations Peripheral blood chimerism analysis post-transplant	peripheral blood that persisted at 20 weeks post-transplant, while leukocytes, red blood cells, and platelet counts remained unaltered.
Suryadinata et al., 2019, Indonesia (264)	To examine the effects of exposure duration to VP aerosol exposure on the levels of antioxidant superoxide dismutase and malondialdehyde in blood of wistar rats	Rats/Wistar Male (n=5) 2-3 months old	Exposure: whole body inhalation Groups: PG/VG/N, Air VP: NR PG/VG ratio: NR Nicotine: 6mg Flavour: NR VP Settings: NR Regime: 5 groups with different time exposure to VP aerosol: 5min/day for 1 week, 2 x 5min/day for 1 week, 5min/day for	Levels of superoxide dismutase and malondialdehyde in the blood	VP exposures reduced blood levels of antioxidant superoxide dismutase ($p<0.005$, in all the treatment groups) and increased levels of malondialdehyde ($p<0.005$, in all the treatment groups) Changes between superoxide dismutase and malondialdehyde levels showed a strong ($r = 0.893$) and significant ($p = 0.000$) relationship

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			2 weeks, 2 x 5min/day for 2 weeks, 5min/day for 3 weeks		
Other					
Studies comparing effects of VP, TC and air exposure					
Cobb et al., 2018, US (265)	To assess the stress induced cellular damage caused by VP exposure using the nematode Caenorhabditis elegans	Nematode Caenorhabditis elegans/ N2 Bristol wild-type	Exposure: whole body inhalation Groups: PG/VG/N, TC, Air VP: eGo. Jomo Tech PG/VG ratio: 1:1 Nicotine: 20mg/mL Flavour: NR VP Settings: 3.7V, 2.6Ω Regime: 15, 30 or 45 cycles of a 5s puff with 10s intervals (2.8mg nicotine/15 puffs) TC: Marlboro, same regime	Movements, survival and stress-induced sleep for up to 24h after exposure Relative expression levels for metallothionein genes (mtl-1 and mtl-2) 1, 5, and 24 h after exposure	Nematodes exposed to TC, but not VP, underwent stress-induced sleep in a dose dependent manner. Increased mtl-1 expression in a dose and time dependent manner in TC group only, with a maximum expression at 5h post exposure. No difference in mtl-2 expression levels in response to either TC smoke or VP aerosol.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Conklin et al., 2018, US (266)	To examine urinary metabolites of 4 common aldehydes in VP exposed mice and to evaluate their potential utility as biomarkers of exposure	Mice/C5BL6/J Male (n=3-5) 12-20 weeks old	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG/N/F, TC, Air VP: blu PG/VG ratio: NR Nicotine: 13-16mg/ml Flavour: tobacco or menthol VP Settings: 3.7V, 1.8Ω Regime: 91ml/4s/puff, 2puffs/min, 13 cycles of 18puffs (equivalent to 1cig) per 4 hours TC: 3R4F, 35ml/puff, 2s/puff, 1puff/min, 9puffs/cig, 2cig/hour, 12cig/day for 6 hours	Urinary nicotine and nicotine metabolites (cotinine, COT; trans-3'-hydroxycotinine, 3HC) and the 3HC/COT ratio (marker of CYP2A6 activity) Urinary metabolites of two saturated aldehydes, formaldehyde (formate) and acetaldehyde (acetate), and two unsaturated aldehydes, acrolein (3-hydroxypropyl mercapturic acid, 3HPMA) and crotonaldehyde (3-hydroxy-1-methylpropylmercapturic acid, HPMMA)	Both TC and VP exposure increased urine levels of formate, acetate and 3-HPMA (menthol VP, but not tobacco VP), while HPMMA levels were only increased in TC group. Levels of nicotine and its metabolites were higher in the urine of TC vs. both VP-exposed groups Urinary 3-HPMA and sum of nicotine metabolites were increased following exposure to menthol-flavoured VP aerosol compared to tobacco-flavoured VP ($p<.05$).

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Reumann et al., 2020, Germany (268)	To examine the effects of VP aerosols and TC on bone morphology, structure, and strength following 6-month inhalation in mice	Mice/ApoE-/- Female (n=6-10) 8 weeks old	Exposure: whole body inhalation Groups: PG/VG, PG/VG/N, PG/VG/N/F, TC, Air VP aerosol: generated via capillary aerosol generator (by PMI) PG/VG ratio: 3:7 Nicotine: 4% (36mg/mL) Flavour: none VP Settings: NR Regime: 3h/day, 5days/week for up to 6 months TC: 3R4F, 55ml/puff, 1puff/30s, 10-11puffs/cig (matched nicotine concentration, 35µg/L), 3h/day, 5days/week for up to 6 months	General bone characteristics: body weight, tibial weight and tibial length Analysis of bone structure (total and cortical bone architecture, including bone volume and bone area fractions), bone biomechanical stability (stiffness and ultimate load) and morphology Nicotine, cotinine, PG, total nicotine metabolites, CoHb levels in plasma, serum and urine (at 3 and 6 months)	Neither cortical bone structure nor biomechanical parameters were compromised in VP exposed groups. In contrast, TC exposure caused a decrease in cortical and total bone volume fraction and bone density relative to other VP groups (especially PG/VG) but not control group. TC exposed group also showed a decrease in ultimate load ($p<.05$ vs. PG/VG and $p<.01$ vs. PG/VG/N and PG/VG/N/F) and stiffness ($p<.05$ vs. control and PG/VG/N/F). No difference in body weight, tibia bone weight or length among the groups. Bone morphology analysis revealed microcracks in cortical bone areas in both TC and VP exposed groups.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Troiano et al., 2019, US (267)	To evaluate the rate of flap necrosis in VP aerosol-exposed rats and to compare it with that of TC smoke-exposed animals	Rats/Sprague Dawley Male (n=15)	Exposure: whole body inhalation exposure Groups: PG/VG/N, TC, Air VP: blu PG/VG ratio: NR Nicotine: 24mg/mL Flavour: NR VP Settings: NR Regime: 2x30min/day, for 30 consecutive days TC: Marlboro, (matched serum cotinine levels, 150-200ng/ml), same regime Random pattern dorsal skin flaps were raised at day 30	Body weight Serum cotinine levels Percentage of flap necrosis (ratio of necrotic tissue to total flap area)	Higher percentage of flap necrosis in both VP (95% CI, 59.9-71.8; P < .001) and TC (95% CI, 64.3-73.0; P < .001) exposed animals compared to control group (95% CI, 46.0-55.6; P < .001) No difference between VP and TC exposed groups (95% CI, 59.9-71.8 vs. 95% CI, 64.3-73.0; P = .46)

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
Studies comparing effects of VP and air exposure					
Javadi-Paydar et al., 2019, US (269)	To examine nicotine-typical effects on spontaneous locomotion and thermoregulation in rats following exposure to VP containing nicotine and tetrahydrocannabinol (THC)	Rats/ Sprague Dawley Male (n=8) 14-15 weeks old	Exposure: whole body inhalation Groups: PG alone or with nicotine (PG/VG/N) and/or THC (12.5 or 25mg/ml) VP: Protank 3 Atomizer, MT32 coil, Kanger Tech PG/VG ratio: NR Nicotine: 1, 10 or 30mg/mL Flavour: NR VP Settings: 2.2Ω Regime: 4x10s puff with 2s intervals every 5min for 30min or for 4 hourly 15-min sessions	Body temperature and activity rate evaluated 30-210min post 30-min exposure or during 270min of repeated inhalations (4 hourly 15-min exposures) Plasma nicotine and cotinine	30-min inhalation of the PG vehicle or nicotine (1, 10 and 30mg/ml) reduced locomotor activity and body temperature of rats with the lowest values in 30mg/ml nicotine group ($p<.05$ vs. baseline, PG, 1 and 10mg/ml nicotine). Repeated inhalations also showed a nicotine related hypothermic response, while spontaneous locomotor activity was consistently increased relative to baseline and PG group. These effects were attenuated (temperature) or blocked entirely (locomotion) by pre-treatment with mecamylamine (nAChR antagonist). In the combination condition, THC suppressed nicotine-induced locomotor activity and led to additional decrease in

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
					nicotine-induced temperature.
Lefever et al., 2019, US (270)	To determine the effects of route of administration and VP aerosol formulation on the discriminative stimulus effects of nicotine in mice	Mice/C57BL6 Male and Female (n=6-8), trained to discriminate 0.75mg/kg subcutaneous (s.c.) nicotine from saline.	Exposure: whole body inhalation Groups: PG/VG/N, s.c. nicotine VP: iStick ELeaf, CE5-S tank Aspire PG/VG ratio: 1:1 Nicotine: 12, 18, 24 or 20mg/mL Flavour: menthol VP Settings: 1.8Ω, 7W Regime: 10s/puff, 5-min exposure s.c. nicotine	Stimulus substitution tests to evaluate the degree to which aerosolized VP containing nicotine substituted for injected nicotine Nicotine and cotinine in plasma and brain	Aerosolized VP with nicotine, regardless of formulation, produced concentration-dependent increases up to maximum of 46–62% nicotine-associated responding Both brain and plasma nicotine concentrations for each sex were similar for s.c. (0.75 mg/kg) and aerosolised (30mg/ml) nicotine with greater levels in females compared to males ($p<.05$). Cotinine levels were similar across sex, but greater levels were found after aerosol exposure compared to s.c. ($p<.05$).
Shao et al., 2019, US (271)	To develop a chronic intermittent VP aerosol delivery method for rodents that simulates human VP use	Mice/ApoE-/- Male (n=5) 8 weeks old WD (40% of calories from fat, 43% from carbohydrat	Exposure: whole body inhalation Groups: PG/VG/N/F, Saline VP: NR PG/VG ratio: NR Nicotine: 2.4% Flavour: tobacco	Body weight, cumulative food intake and locomotor activity Plasma nicotine and cotinine levels to match human exposure	Chronic VP exposure decreased body weight, food intake and increased locomotion ($p<.05$) in ApoE−/− mice

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
		e, and 17% from protein)	VP Settings: NR Regime: Dose-response (4 exposure regimes) and time-course (5 time points) experiments to match nicotine exposure in vapers, the selected protocol for chronic exposure: 4s/puff, 8puffs with 25s intervals every 30min during the 12-h dark phase, for 12 weeks		
Wetendorf et al, 2019, US (272)	To examine the effects of VP on pregnancy initiation and second-generation fetal reproductive health	Mice/C57BL6	Exposure: whole body inhalation using Scireq Inexpose system Groups: PG/VG/N, Air VP: eVic VTC Mini Joyetech PG/VG ratio: 55:45 Nicotine: 24mg/mL	Fetal outcomes of VP aerosol exposed dams before and during pregnancy	VP delayed the onset of the first litter ($p<.002$ vs. air-control), with no delay in future pregnancies. Also, VP exposed animals exhibited a delay in embryo attachment, as evidenced by the absence of implantation sites at day 5.5 despite high progesterone levels, which indicated pregnancy.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			Flavour: none VP Settings: 0.15Ω Regime: 2s/puff, 2puffs/min, 3h/day, 5days/week, for 4 months		VP maternal inhalation misregulated receptive signaling pathways (767 dysregulated genes) at implantation, including major pathways important for uterine receptivity. Male offspring exposed to VP in utero exhibited an insignificant impairment of fertility, while female offspring had significantly lower body weight in adulthood (p<.006 vs. air-control).
Montanari et al., 2020, US (273)	To test concentration-dependent effects of nicotine VP inhalation on blood-nicotine and cotinine concentrations, and somatic withdrawal signs over time in rats	Rats/ Wistar Adult Male (n=6)	Exposure: whole body inhalation Groups: PG/VG/N VP: KangerTech PG/VG ratio: 1:1 Nicotine: 20, 40 or 80mg/mL Flavour: NR VP Settings: 2.2Ω, 4.5W Regime: 3s puff every 2 min, 30puffs in 60min per day, for 11 consecutive days	Spontaneous and precipitated (mecamylamine-induced) somatic withdrawal signs (1, 2, 4, 6 and 24 hours post exposure on day 10 and 11, respectively) Dose and time course of plasma nicotine and cotinine levels (1, 15, 30, 60 and 120 min post exposure on day 1 and 10)	Exposure to nicotine VP produced time- and dose-dependent somatic withdrawal signs with higher withdrawal scores obtained following precipitation of withdrawal with systemic mecamylamine injection. Blood nicotine and cotinine levels changed in a time- and dose-dependent manner following exposures to nicotine VP.

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			On day 11, Injected with mecamylamine 1h post exposure		
Henderson et al., 2021, US (274)	To examine the alteration in self-administration behaviour and plasma cotinine levels following exposure to nicotine salts and VP	Mice/C57BL6 Male and female (n=7-8) 3 months old	Exposure: eVape self-administration (EVSA) Groups: PG/VG, PG/VG/N, VP: SMOK baby beast TFV8 X-baby Q2 tank PG/VG ratio: 1:1 Nicotine: 6mg/mL Flavour: none VP Settings: 0.4Ω, 65W Regime: Mice were escalated on a fixed ratio 1 (FR1) schedule in 10 daily 2h sessions (5d/week), 3s/puff with 30s interval following a nose-poke, trained to acquire EVSA	Plasma cotinine Self-administration behaviour (Mean FR3 EVSA deliveries for male and female)	Both male and female mice assigned PG/VG/N exhibited increased EVSA on a FR3 schedule compared to PG/VG ($p<.01$ and $p<.05$, respectively). Exposure to nicotine salts produced the highest FR3 deliveries ($p<.001$ and $p<.05$ vs. PG/VG, $p<.01$ and $p<.001$ vs. PG/VG/N for male and female mice, respectively). Both male and female mice assigned to nicotine salts produced higher plasma cotinine levels compared to PG/VG/N ($p<.05$ and $p<.01$)

Authors	Study aims	Animal model (n per group)	Exposure method and dose characteristics	Biomarkers and outcomes assessed	Main outcomes (vs. control unless otherwise stated)*
			behaviour on 6mg/ml nicotine with 15mg/ml menthol, PG/VG/N/F. Then transitioned to a FR3 for 5 days: PG/VG, PG/VG/N (with 6mg/ml nicotine), and 6mg/ml nicotine-salt, each for 4 days, followed by re-baseline on day 5 with PG/VG/N/F		

Notes: * The reported findings are significant and compared to air-control group unless otherwise indicated.

AA – acetaldehyde; ACE-2 – angiotensin-converting enzyme 2; BALF – bronchoalveolar lavage fluid; BBB – blood-brain barrier; CPu – caudate-putamen; Crf – corticotropin-releasing factor; CRP – C-reactive protein; CYP – cytochrome P450; DBP – diastolic blood pressure; ERK1/2 – extracellular signal-regulated kinase 1/2; EVALI – e-cigarette or vaping product use-associated lung injury; FA – formaldehyde; FC – frontal cortex; GLT-1 – glutamate transporter-1; GTT – glucose tolerance tests; HDL-C – high-density lipoprotein cholesterol; HIP – hippocampus; HR – heart rate; HSC – hematopoietic stem cells; IgM – immunoglobulin M; ILs – interleukins; JNK – cJun NH(2)-terminal kinase; LDH – lactate dehydrogenase; LDL-C – low-density lipoprotein cholesterol; LPS – lipopolysaccharide; MBP- mean blood pressure; MDA – malondialdehyde; MPO – myeloperoxidase; nAChR – nicotinic acetylcholine receptor; NNK – 4-(methylnitrosamino)-1- (3-pyridyl)-1-butanone; NR – not reported; nnVP – non-nicotine vaping product; P – postnatal day; PG – propylene glycol; PG/VG/N/F—exposure to aerosol containing propylene glycol, vegetable glycerine, nicotine and flavourings; PMI – Philip Morris International; PWV – pulse wave velocity; SBP – systolic blood pressure;

RBC – red blood cells; ROS – reactive oxygen species; s.c. – subcutaneous; STR – striatum; TC – tobacco cigarette; TCh – total cholesterol; TG – triglyceride; TJs – tight junctions; TNF – tumor necrosis factor; VG – vegetable glycerine; VP – vaping product; WBC – white blood cells; xCT – cystine/glutamate antiporter.

Appendix 7. Table 7: a summary of in vitro (cell) studies evaluating health effects of vaping products inhalation exposure

The reported findings are significant and compared to control group unless otherwise indicated.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
Respiratory				
Studies reporting adverse effects of VP, HTP and TC exposure				
Leigh et al., 2018, US (275)	To examine the potential toxic effects of inhaling emissions from HTP in comparison with VP and TC	Human bronchial epithelial cells (H292) at the ALI	VP aerosol (MarkTen), tobacco flavour with 3.5% nicotine, 55 puffs HTP (IQOS), tobacco flavour, 12 puffs TC (Marlboro Red), 8 puffs 55ml/2s/puff, matched nicotine delivery	TC exposure was more cytotoxic than HTP, VP or air-control, while HTP showed higher cytotoxicity compared with VP and control. Increased cytokines levels (IL-1β and IL-6) were found following TC exposure, but not VP and HTP.
Delaval et al., 2019, Switzerland (276)	To evaluate the adverse effects of VP exposure on normal human airway epithelia in comparison to TC	Human bronchial epithelial cells at the ALI	VP (Joyetech, SmOkay) and HTP (IQOS), 75mL/3s/puff with 22 s intervals, 21 puffs TC smoke (3R4F), 6cig simultaneously, 35mL/2 s/puff, 1 puff/cigarette/min, 8.45 min Assessed 0h, 4h and 24h	A single exposure to aerosols generated from the 2nd generation (SmOKay) VP induced higher cytotoxicity (measured by LDH assay) than those of the 4th generation (Joyetech) VP, while there was no effect by the HTP product and TC. Also, subtle, VP-dependent

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
			after exposure	changes of cell morphology were noted
Dusautoir et al., 2021, France (277)	To compare cytotoxicity, oxidative stress and inflammatory response in human bronchial epithelial cells following exposure to HTP, VP aerosols and TC smoke	Human bronchial epithelial cells (BEAS-2B) at the ALI	Aerosols from two VP models (Lounge, 4.6W, and ModBox, 18W and 30W), blond tobacco flavoured 65%PG/ 35%VG with 16mg/mL nicotine, 40-120puffs HTP (IQOS 2.4 model), 12 puffs/heat-stick, 2-40puffs TC (3R4F), 10puffs/cig, 1-10 puffs 55ml/2s/puff every 30s for all products	Exposure to VP aerosol resulted in no (Modbox) or low (Lounge box, >75%) cytotoxic effect (assessed by ATP content). With a strong reduction of cell viability from 12puffs, HTP emissions exhibited increased cytotoxicity (2% at 120 puffs) compared with VP aerosols but lower than TC smoke (2% at <10 puffs). HTP and VP induced oxidative stress (increased CYP1A1, CYP1B1, HMOX1, NQO1 expression for all products) and inflammatory response (changes in IL6, IL8, MCP-1 and GROα) in a manner similar to that of TC smoke, but after more intensive exposures.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
Studies reporting adverse effects of VP vs. TC, VP vs. HTP or HTP vs. TC				
Banerjee et al., 2017, UK (278)	To compare transcriptomic perturbations and cytokine profile in MucilAir, a commercially available lung epithelial tissue, after short repeated exposure to TC and VP aerosols.	3D human airway epithelia culture system MucilAir at the ALI	Aerosol from VP (Vype ePen), blended tobacco with 18mg/mL nicotine, or TC smoke (3R4F) Both with 1:20 (aerosol:air, v:v) dilution, 55ml/2s puff every 30s, 4 x 5min with 30min intervals Analysis 24 and 48h post-exposure	VP exposure caused a limited impact on the release of inflammatory mediators (only 1 cytokine was downregulated at 48h) and transcriptomic profile, albumin (ALB) and SP140 were confirmed as differentially expressed. In contrast, TC exposure caused a strong inflammatory response (8 out of 33 cytokines were upregulated, $p<.05$) and perturbations in xenobiotic metabolism, oxidative stress response Exposure to VP and TC did not cause cytotoxicity.
Fields et al., 2017, US (279)	To assess exposure parameters of the VITROCELL VC1 smoking machine and evaluate donor-to-donor variability of three EpiAirway tissue donors with the following endpoints: viability,	The EpiAirway model of primary human tracheal/bronchial epithelial cells at the ALI	VP aerosol (MarkTen Classic and NJOY Bold), 55ml/3sec/puff every 30sec, 90 puffs/cartridge, 20-180 puffs. TC (3R4F), 55ml/2sec/puff every 30sec, 11 puffs/cig, 11 – 176 puffs	VP aerosol yielded no impact on cellular toxicity, tissue integrity and promoter regulation of a key controlling pathway of oxidative stress. In contrast, TC exposure yielded a dose-dependent decrease in cell viability and barrier function and increased nuclear factor

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	barrier integrity, and gene promoter/expression regulation.			erythroid 2-related factor 2 (Nrf2) promoter activation through the antioxidant response element and gene expression associated with oxidative stress, inflammation, and metabolism.
Adamson et al., 2018, UK (280)	To characterise the use of the puffing system (LM4E) for the in vitro assessment of VP and HTP aerosols.	Human adenocarcinoma lung epithelial cells (H292) at the ALI	Undiluted aerosol from VP: tobacco flavoured 18mg/ml with nicotine, 55ml/3s puff every 30s, 60, 120 and 240 puffs (0.5-2h) Undiluted aerosol from HTP: 55ml/2s puff every 30s, 3-120puffs (1.5-60 min) Using the Borgwaldt LM4E puffing machine (4.0V, 2.8Ω)	Complete cytotoxicity was achieved after 1h exposure to HTP, while 2h exposure to VP aerosol resulted in 21.5 ± 17.0% cell survival (using neutral red uptake assay). Positive correlation between puff number and nicotine concentration in the media after both exposures.
Vasanthi Bathrinarayanan et al., 2018, UK (281)	To evaluate VP cytotoxicity using a physiologically relevant in-vitro multicellular model of human airways	Human bronchial epithelial cells (CALU-3) and pulmonary fibroblasts (MRC-5) co-cultured at the ALI	VP aerosol, strawberry flavoured with 16mg/ml nicotine, 1h-6h TC (Marlboro red), for 7 min 35ml/2s/puff every 60s, each exposure was followed by 24h incubation	VP caused elevated IL-6 and IL-8 (1h-6h) and increased oxidative stress (H2O2 levels, 3h), but the levels were lower than after TC. 7-min TC exposure reduced cell viability, while VP decreased cell viability only after 3h. TC (7min), but not VP (3h),

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
				exposure induced the expression of pro-apoptotic mediators, namely caspase 3/7.
Czekala et al., 2019, UK (282)	To examine the biological effects of nicotine-containing VP in comparison to TC	3D EpiAirway tissues at the ALI	VP aerosol using VITROCELL VC1, blu PLUS, 55.8%PG/ 39% VG, 24mg/mL nicotine with or without blueberry flavouring, 55ml/3s/puff, 30s intervals, 80, 240, or 400 puffs TC smoke generated using a VITROCELL VC1, 55ml/2s/puff, 30s intervals, 9, 27 or 45 puffs (equivalent to 1, 3 or 5 cig) Analysed 24h after exposures	While little or no effect was observed after exposure to VP aerosol with or without flavouring, TC exposure decreased tissue viability and barrier function, increased secretion of inflammatory cytokines (interleukin 6 and 8, IL-6 and IL-8) and oxidative stress marker (8-isoprostane), as well as altered a marker of DNA damage (γ-H2A histone, γ-H2AX).
Iskandar et al., 2019, Switzerland (283)	To compare the biological impact of an acute exposure to VP aerosols of different compositions and TC smoke using human organotypic buccal and small airway cultures	3D Human organotypic buccal (EpiOral) and small airway epithelial (SmallAir) cultures at the ALI	Aerosol generated from PG/VG alone or with nicotine (PG/VG/N) and flavours (PG/VG/N/F) (flavour type not reported) MarkTen device, 55ml/5s/puff every 15s 4puffs/min for 28min, 112 puffs	Exposure to TC caused apparent tissue damage in buccal and small airway cultures and reduced the frequency of cilia beat in small airway cultures, while VP exposures of different compositions had no impact on morphology of both tissue cultures.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
			TC smoke (two 3R4F), 55ml/2s/puff every 15s for 28min (10cig), 112puffs at different dilutions (69%, 24% and 13%) Analysed pre- and post-exposure (0h, 2h, 24h, 48h)	VP exposures triggered alterations in gene expression and the profiles of secreted inflammatory mediators, but at a lower extent than that observed following TC exposure. There was no difference between different VP compositions, but molecular and cellular changes were tissue type-specific.
Ghosh et al., 2020, US (284)	To determine deterioration of epithelial cell barrier from sub-chronic exposure to TC smoke, VP aerosol and tobacco waterpipe exposures (TW)	Human bronchial epithelial cells (hBEC) at the ALI for TC and VP exposures Pseudostratified primary epithelial tissue (MucilAir) for TW exposures	Aerosol from tobacco flavoured e-liquid with 0 or 12mg/mL nicotine, 55ml/3s/puff with 30s intervals, 10 puffs/day for 10 days TC (3R4F), 55ml/3s/puff every 30s, 1cig/day (10puffs) for 10 days TW, 530ml/3s/puff every 17s, 1h/day with 48h intervals, 3 exposures Analysed 16-18h post-exposure	Nicotine containing VP substantially decreased airway epithelial barrier function similar to TC exposure: both TC and VP with nicotine reduced TEER to 49 and 60%, reduced ciliary beat frequency to 62 and 59%, and cilia moving to 47 and 52%, as well as increased cell velocity by 2.5 and 2.6 times, respectively. Also, exposure to TC alone increased monolayer permeability by 6-fold and dismantled adherence junction as reflected by reduced expression of E-cadherin (by 39%). nnVP and TW exposures resulted in more moderate decreases in epithelial integrity.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
Herr et al., 2020, Germany (285)	To compare the acute effects of TC and VP exposure on host defense, inflammation, and cellular activation of cell lines and primary differentiated human airway epithelial cells	Human lung adenocarcinoma cell line (Calu-3) Human bronchial epithelial cell line (NCI-H292) Primary human bronchial epithelial cells (hBEC) Cultured at the ALI	Aerosol from 60%PG/ 30%VG/ 10%water with 18mg/mL nicotine, 3s/puff every 29s for 15min TC (Marlboro), 3cig in 15min (matched nicotine concentration)	VP exposure had no effect of the bacterial count, barrier integrity and the expression of antimicrobial peptides after infection with P. aeruginosa in Calu-3 cells. In contrast, TC negatively affected host defence and reduced barrier integrity. Furthermore, VP-exposure induced IL-8 secretion from Calu-3 cells but not NCI-H292 or hBEC, and stimulated transcription of other inflammatory markers like S100A7 and S100A12 in hBEC, but to a lower extent compared to TC exposure. Distinct transcriptome patterns of host defence and inflammatory genes between TC- and VP-exposed cells.
Rouabhia et al., 2020, Canada (286)	To evaluate the effect of VP aerosol on healthy human nasal epithelial cell viability and growth, and to assess the possible adverse effects of VP on	Human primary nasal epithelial cells and engineered 3D nasal mucosa tissues at the ALI	Aerosol from 50%PG/ 50%VG without or with 18mg/mL nicotine, 5-6s/puff every 25-30s, 2puffs/min TC (3R4F), 0.7mg of nicotine/cig, half of cig per exposure	VP-exposed nasal epithelial cells displayed morphological changes, including a larger cell size and a faint nucleus, and decreased cell viability (LDH activity). Tissues exposed to VP aerosol displayed a structural deregulation, with more large-

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	nasal tissue structure and pro-inflammatory cytokine release		2 x 15 min per day, for 3 days	sized cells, fewer Ki67-positive cells, and a reduced nasal cell growth. Cytokine measurements showed high levels of IL-6, IL-8, TNFα, and MCP-1, demonstrating VP-induced pro-inflammatory cytokine responses. These effects were greater in TC-treated cells than with VP.
Wieczorek et al., 2020, UK (287)	To examine the cytotoxic, genotoxic and mutagenic responses of two commercial VP devices when compared to TC smoke	Human hepatocellular carcinoma cells (Hep-G2) Human bronchial epithelium cells (BEAS-2B) at the ALI	BEAS-2B was exposed to both e-liquid and VP aerosol, while Hep-G2 exposced to e-liquid only. 12 e-liquids and corresponding aerosols, 12 and/or 24mg/mL nicotine with the following flavours/flavour descriptors: tobacco, gold leaf, menthol, mint chocolate, vanilla, caramel cafe, cherry, strawberry mint, berry cobbler, blueberry, glacier mint or caroline bold flavourings, using blu GO™ disposable and blu PLUS+™ rechargeable 50%PG/50%VG with 12 or 24mg/mL nicotine	TC smoke induced a substantial increase in cytotoxicity (neutral red uptake assay), mutagenicity and genotoxicity in all the cells (assessed by bacterial reverse mutation and in vitro micronucleus assays, respectively). In contrast, there was no mutagenic or genotoxic effects when either VP liquids or corresponding aerosols were tested. Differential cytotoxic responses depending on nicotine concentration, cell type, flavourings and device were observed after VP exposures, but to substantially lower extent compared to TC exposure. Overall, 24mg/ml nicotine

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
			TC(3R4F) 55ml/2-3s/puff every 30s	products were more cytotoxic compared to 12mg/ml nicotine. Aerosol generated from blu GO™ device appeared to be more cytotoxic than that from blu PLUS+™ device. Hep-G2 cells were found to be 30% more sensitive to the effect of e-liquid exposure than BEAS-2B cells.
Palazzolo et al., 2017, US (288)	To test the effect of VP aerosol and smoke on mucous transport velocity using the frog palate paradigm.	Bullfrog palates	VP aerosol from 80% PG/ 20% VG with 24mg/mL nicotine, 3.4mg/15puffs, 45 cycles of 5s puff with 10s intervals TC smoke (Marlboro), 1mg of nicotine/cig, 15puffs/cig	VP aerosol had a modest inhibitory effect ($p < 0.05$) on mucous transport velocity (MTV) 1-day post-exposure and VP aerosol sedimentation accounted for epithelial thickening. In contrast, TC smoke completely inhibited MTV immediately after exposure and the MTV was unable to recover 1 day later, which was, in part, due to decreased number of cilia and disruption of the TC-exposed epithelium.
Vermehren et al., 2020, Germany (289)	To compare the effects of VP aerosol and TC smoke on human gingival fibroblasts in terms of	Human gingival fibroblasts (HFIB-G)	Aerosol from passion fruit / peach flavoured 55%PG/35%VG/10% water with 3mg/ml nicotine, 70ml/4s/puff every 30s, for 15min	Decreased cell proliferation was only observed 24h post-exposure to TC, while there was no difference between groups after 48 and 72h.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	proliferation, metabolic activity, cell death and formation of reactive oxygen species (ROS)		TC (Marlboro), 35ml/2s/puff, for 15min Analysed 1h, 24, 48 and 72h post-exposure	Increased metabolic activity of fibroblasts was obtained 1h and 48h post-exposure to VP compared to TC and control groups. After 24h, both VP and TC-exposed cells showed increased metabolic activity. Increased ROS formation 1h, 3h and 6h post-exposure to TC compared to VP and control groups. No difference regarding caspase 3/7 activation or the amount of apoptosis/necrosis among the groups.
Giralt et al., 2021, Switzerland (290)	To compare the biological impact of VP aerosol (IQOS MESH) and TC smoke on human bronchial and alveolar cultures	Human organotypic bronchial epithelial culture (MucilAir) and alveolar triculture models (human alveolar epithelial (A549), macrophage-like (differentiated THP-1) and endothelial (EA.hy 926)□ cells) at the ALI	A cartridge VP (IQOS MESH) aerosol using classic tobacco liquid, 55ml/3s/puff every 30s, 4 puffs/min for 1, 7 or 28min TC (3R4F), 3-31% concentration, 55ml/2s/puff every 30s, 4puffs/min for 7 (28puffs) or 28min (112puffs)	Aerosol generated with the cartridge VP did not cause cytotoxicity in bronchial epithelial cultures or alveolar tricultures and had a smaller biological impact compared to TC. TC exposure caused a dose-dependent reduction in cell viability, marked decrease in the frequency and active area of ciliary beating and altered levels of various mediators (VEGFA,

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
				TIMP1, MMP-9, MMP-1, and IL–8) in bronchial cultures. Global mRNA expression and secreted protein profiles revealed a lower impact of VP aerosol exposure than TC exposure.
Tellez et al., 2021, US (291)	To evaluate the cytotoxicity and genotoxicity of VP aerosols containing diverse flavouring products with and without nicotine in oral epithelial cell lines	Immortalised oral epithelial cell lines (MOE1A, MOE1B, MSK-LEUK1)	VP aerosols using 10 flavoured 70% PG/30%VG without and with 12mg/mL nicotine; flavour brands included Arctic Blast, Blue Pucker, Jamestown, Love Potion, Mardi Gras, Midnight Splash, Port Royale, Tobacco Row, Tortuga, and Uptown. Unflavoured 70%PG/30%VG without or with 12mg/ml nicotine TC(3R4F) 52ml/2.6s puff every 18s for 20min, dose range 150-450 mg TPM/m3 for VP, 100-400mg TPM/m3 for TC	Three flavoured VP aerosols caused ≥20% cell toxicity (neutral red uptake assay) and lipid peroxidation. Nine flavoured VP products induced oxidative stress levels up to 2.4-fold (ROS-Glo assay) in at least 1 cell line, with dose response seen for nicotine-containing Love Potion across all cell lines. Genotoxicity, as evidenced by micronuclei formation, was increased up to 5-fold for some products with Blue Pucker being the most genotoxic VP. While PG/VG exposure led to micronuclei formation, it did not show any other significant effect. TC exposure induced cytotoxicity of 65-75% and caused dose-dependent induction of oxidative

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
				stress and DNA damage with maximum fold increases for lipid peroxidation in all cell lines. Micronuclei were also formed in response to TC exposure.
Manyanga et al., 2021, US (292)	To investigate whether VP aerosol exposure alters cisplatin response in head and neck cancer cells	Human epithelial cancer cell lines from different head and neck regions UM-SCC-1 (floor of mouth), WSU-HN6 (tongue), and WSU-HN30 (pharyngeal)	VP aerosol extracts with and without nicotine or TC extract added directly to the culture media (0-39ng/ml nicotine) for 48h VP exctract-treatment was followed by VP extract with cisplatin (0.01-100µM) for another 48h	Exposure to VP aerosol extract during cisplatin treatment reduced cancer cell death, increased cell viability and clonogenic survival, a marker of unlimited reproductive viability of the surviving cells. Exposure to VP aerosol extracts increased the dose of cisplatin required to induce a 50% reduction in cell growth (IC50) in a nicotine-independent manner in all HNSCC cell lines tested. VP exposure also altered the expression of DNA damage repair genes and the expression of drug influx and efflux transporters. TC extracts induced similar increases in cisplatin resistance.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
Studies reporting adverse effects of VP exposure				
Ghosh et al., 2018, US (134)	To determine the effects of chronic VP exposure on pulmonary epithelia	Human bronchial epithelial cultures (hBEC) at the ALI	Aerosolised 55% PG/45% VG, 36 puffs/day, for 1 or 4 days	Exposure to PG/VG increased intracellular MUC5AC levels (3-4 fold) and CYP1B1 expression, as well as affected plasma membrane fluidity and protein diffusion.
Chung et al., 2019, US (225)	To test the effects of nicotine-containing VP on airway mucociliary function in human bronchial epithelial cells	Primary human bronchial epithelial cells (hBEC) from never-smokers at the ALI	VP aerosol using Joyetech eVic, 50%PG/50%VG without or with 36mg/ml nicotine	VP exposure reduced airway surface liquid hydration and increased mucus viscosity of hBEC in a nicotine-dependent manner.
Cirillo et al., 2019, Italy (293)	To investigate the effect of the VP aerosols generated by different coils on the viability of H1299 human lung carcinoma cells	Human lung carcinoma cells (H1299) at the ALI	Aerosol using Eleaf Pico, 3.5V, 1.5 Ω and 0.25 Ω (Joyetech), 50%PG/50%VG without or with 18mg/ml nicotine 4s/puff, 15 puffs with 26s inverval (7.5min exposure), repeated after 2h	VP exposure reduced the viability of H1299 cells by up to 45.8% (measured by MTT assay) with the higher toxicity observed with 0.25Ω coil compared to 1.5Ω, and this effect was inversely related to ROS production in cell media.
Clapp et al., 2019, US (294)	To investigate whether a common flavouring agent cinnamaldehyde disrupts mitochondrial function and impairs ciliary beat	Primary human bronchial epithelial cells (hBEC) at the ALI	Cinnamaldehyde-containing, nicotine-free e-liquids and corresponding aerosols ('Kola, Hot Cinnamon Candies and Sinicide') with 55%PG/45% VG. Box Mod VP (Volcano with a SMOK TFV4 Mini	Exposure to one of three VP aerosols containing cinnamaldehyde (Sinicide only) as well as the corresponding e-liquid rapidly yet transiently suppressed airway cilia motility, which is essential in mucociliary clearance, as compared with the

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	frequency (CBF) on well-differentiated human bronchial epithelial (hBE) cells		Tank). Either 1-15 mM cinnamaldehyde or nicotine (0.5mg/ml) alone, or a mixture of both	PG/VG vehicle. Exposure to different concentrations of cinnamaldehyde alone impaired mitochondrial respiration and glycolysis in a dose-dependent manner and reduced intracellular ATP levels.
Lin et al., 2019, US (295)	To determine if VP aerosol has the potential to induce ion transport abnormalities in the airways, including cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction	Primary human bronchial epithelial cells (hBEC) and Calu-3 cells at the ALI	Tobacco flavoured 100% VG with 12mg/mL nicotine e-liquid (Red Oak Domestic) or 100% VG alone were aerosolised (8s puff, 1-5min or 15-60 min), nebulised (15-60min using 1-2 ml) or added directly to the cell culture (1-300µl). Assessed 1h after exposure	Vaporised e-liquid and VG inhibited chloride ion transport in a dose-dependent manner in Calu-3 cells (30min, 57.2% and 14.4% respectively, p<.0001), while unvaporised e-liquids produced no effect. VP vapour also reduced ATP-dependent responses and epithelial sodium channel activity (by 95%) in hBE cells in a dose-dependent fashion, suggesting reduced epithelial ion transport beyond CFTR, even without diminished transepithelial resistance or cytotoxicity.
Escobar et al., 2020, US (296)	To assess the pro-inflammatory and cellular stress effects of the vaped	16HBE cells at the ALI and differentiated human bronchial epithelial cells (hBEC)	Aerosol from PG or VG alone or 55%PG/45%VG mixture, 166ml/4s/puff with 26s intervals for 10min (20	In 16HBE cells exposed to VP aerosols there was an increase in pro-inflammatory cytokines IL-6 and IL-8 levels (40W and 85W,

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	humectants PG and VG on airway epithelial cells		puffs), 40W or 85W Analysed 1h, 2h, and 24h post-exposure	mainly PG and VG) and in cellular stress related markers, such as gene expression of NQO1 (85W only, PG, VG and PG/VG), while only VG increased HMOX1 expression and carbonylated proteins at high wattages. Increased cytotoxicity (via LDH assay) was seen in PG exposure at 85W. Additionally, PG exposure alone caused elevated IL-6 expression, while VG exposure at high wattage increased HMOX1 expression in hBEC.
Ganguly et al., 2020, Sweden (297)	To compare pulmonary molecular effects of two popular mixed fruit flavoured e-liquids with and without nicotine	Primary human bronchial epithelial cells (hBEC) and human type II alveolar cells (NCI-H441 with HULEC-5a) cultured at the ALI to develop bronchial- and alveolar mucosa models	Aerosolised two popular sweet mixed fruit flavoured e-liquids without or with 3mg/mL nicotine, 40ml/3s/puff with 30s interval, 10 puffs, 3 times (alveolar) or 6 times (bronchial) per day	Different patterns of molecular response to VP exposures, suggesting altered expression of markers for pro-inflammation, oxidative stress, tissue injury/repair, alarm anti-protease, anti-microbial defence, epithelial barrier function, and epigenetic modification depending on the flavours, nicotine content, and/or lung models (bronchial or alveolar).
Gellatly et al., 2020, US (298)	To investigate the effect of VP on	Primary small airway epithelial cells at the	Tobacco flavoured 80%PG/20%VG with 0 or	Unlike the nicotine-containing VP, nnVP exposure increased IL6

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	human distal airway inflammation and remodeling	ALI	24mg/mL nicotine, 35ml/4s/puff every min, 15puffs	production, which was coupled with increased levels of intracellular MUC5AC protein as a neutralizing IL6 antibody inhibited the nnVP-induced production of MUC5AC.
Leigh et al., 2020, US (299)	To test in vitro the acute inhalation toxicity of vaporized flavored and unflavored nicotine solutions co-administered with cannabidiol (CBD)	Bronchial epithelial cells (H292) at the ALI	Aerosol from PG only, PG with 1.7mg/mL nicotine, PG with 1.7mg/ml CBD, PG with a mixture of both CBD and nicotine (1.7mg/ml each), flavoured liquid (Easy Rider) diluted with PG, nicotine and CBD (1.7mg/ml each), 55ml/2s/puff every 30s, 55puffs or 30min Analysed 2.5h post-exposure	Exposure to PG alone or with nicotine decreased metabolic activity and increased cytokine levels (IL-10 and IL-1β, respectively), while CBD exposure decreased metabolic activity and cell viability, as well as caused increase in IL-1β, IL-10, CXCL1 and CXCL2 release, with greater effects when compared to PG or nicotine. Importantly, co-exposure of nicotine and CBD resulted in an additive cytotoxic and inflammatory response as compared to other treatments. Observed toxic effects were accentuated by flavourings
Noel et al., 2020, US (300)	To determine the influence of VP design characteristics, such as resistance and voltage, on VP	Human bronchial-epithelial cell line (H292) at the ALI	Aerosol from butter-flavoured or cinnamon-flavoured 50%PG/ 50%VG with 36mg/mL nicotine, 55ml/3s/puff every 30s, 2h/day, for 1 or 3	Exposure to butter-flavoured or cinnamon-flavoured VP aerosol under sub-ohm conditions was cytotoxic, decreased tight junction integrity, increased reactive oxygen species

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	aerosol composition and cellular toxicity		consecutive days under sub-ohm (0.15 Ω) and regular (1.5 Ω) vaping conditions Analysed 24h post-exposure	production, and altered expression of key genes associated with biotransformation, inflammation and oxidative stress (OS). Additionally, increased protein levels of 8-hydroxy-2-deoxyguanosine, an indicator of oxidative DNA damage, was found in cinnamon-flavoured VP aerosol-exposed cells. Notably, OS-mediated damage induced by the cinnamon-flavoured VP aerosol was prevented by the pre-treatment with antioxidant N-acetyl cysteine, confirming the involvement of OS as a toxicity process.
Pinkston et al., 2020, US (301)	To evaluate the in vitro toxicity of JUUL crème brûlée-favored aerosols on human bronchial epithelial cell lines and a murine macrophage cell line	Human bronchial epithelial cell lines (BEAS-2B, H292) and murine macrophage cell line (RAW 246.7) at the ALI	Aerosol from the crème brûlée JUUL pods with 5% nicotine, 5s/puff every 30s for 1h Assessed 24h following exposures	Exposure to VP aerosol decreased cell viability (≥50%), increased nitric oxide (NO) production (≥30%), and expression of iNOS gene in BEAS-2B cells. H292 cells or RAW macrophages responded to VP aerosol with increased production of reactive oxygen species (ROS) by ≥20%, while

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
				the cell viability was not affected. Also, RAW macrophages exposed to VP aerosol displayed decreased NO (≥50%) and down-regulation of the iNOS gene, possibly due to increased ROS. Additionally, VP aerosol dysregulated the expression of several genes related to biotransformation, inflammation and airway remodeling, including CYP1A1, IL-6, and MMP12 in all 3 cell lines.
Serpa et al., 2020, US (302)	To investigate whether VP exposure has the potential to decrease lung function by producing a chronic inflammatory environment and altering immune response to infection	Lung epithelial cell lines (mouse MLE12 and human BEAS-2B) Mouse bone marrow–derived primary macrophages (BMDMs) Primary human bronchial epithelial cells at the ALI	Aerosol from 50%PG/50%VG without or with 18mg/mL nicotine, 6s puff every 30s for 4minutes, 7 consecutive days 100 µM nicotine for 4 minutes/day, 7 days	Exposure to VP aerosol led to apoptosis, secondary necrosis, and necrosis in lung epithelial cell models, while macrophages exposed to VP exhibited apoptotic and inflammatory caspase–mediated cell death. Additionally, VP exposures impaired the ability of macrophages to clear apoptotic cells and pathogens by efferocytosis and phagocytosis, and decreased bacterial clearance when challenged with Streptococcus Pneumonia.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
				These effects were enhanced in the presence of nicotine in the VP aerosol, however, nicotine alone did not show any deleterious effects.
Urena et al., 2020, US (303)	To investigate the cytotoxicity and oxidative stress in normal and cancerous human oral cell lines exposed to VP aerosols	Human oral squamous cell carcinoma cells (SCC-25) and normal human gingival fibroblast cells (HGF-1) at the ALI	VP Aerosols from Lava Flow (strawberry, pineapple, and coconut), Very Cool (blueberry, blackberry, and raspberry), Hawaiian POG (orange, passion fruit, and guava), and American Patriots (tobacco), with 0 and 6mg/ml nicotine. Modifiable VP (SMOKTech AL85 Mod with a TFV8 Baby Tank) 100ml/4s/puff every 26s, 15 puffs/1h for 1h (oxidative stress) or 3h (cytotoxicity)	Only one of eight tested aerosols (Lava Flow with 0mg/mL nicotine) induced cytotoxicity (MTT assay) against two human oral cell lines. Independent of nicotine content, exposure to the cytotoxic Lava Flow caused a concomitant increase in intracellular oxidative stress in both SCC-25 and HGF-1 cells (1.7- to 1.9-fold, respectively). No effect was observed after exposure to the non-cytotoxic Hawaiian Pog. The cytotoxic potential of the aerosols increased as a function of atomizer age (0-900 puffs) with both treatments.
Woodall et al., 2020, UK (304)	To investigate the effects of PG and PG/VG on glucose uptake in proliferating human H441 and primary bronchial airway	Human H441 airway epithelial cells and primary human bronchial epithelial cultures (hBEC) in submerged culture and at the ALI	3% of PG alone or 3% of 55%PG/45%VG applied to medium for 0-24h or cells exposed to corresponding aerosol, 70ml/5s/puff, 27puffs/10min	3% PG/VG puffed onto the apical surface of hBEC decreased glucose transport as indicated by reduced cell height ($p<.001$) and basolateral glucose uptake ($p<.05$).

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	epithelial cells			Direct application of 3%PG or 3% PG/VG (30–60 min) inhibited glucose uptake and mitochondrial ATP synthesis in H441 and hBEC. 3% PG/VG also inhibited glycolysis, compromised barrier function and increased epithelial permeability.
Behar et al., 2018, US (305)	To compare the cytotoxicity of VP fluids/solvents and their corresponding aerosols using in vitro cultured cells	Human pulmonary fibroblasts (hPF) Lung epithelial cells (A549) Pluripotent human embryonic stem cells (hESC)	35 VP e-liquid refills and do-it-yourself products with a range of solvents, nicotine concentrations (0-24mg/mL) and flavours Corresponding aerosols produced using Johnson Creek's Vea, 30ml/4.3s/puff, 10puffs/h, 24puffs/4ml of medium Dose-response experiments using 0.0006–6puffs/mL of medium	74% of fluids accurately predicted the cytotoxicity (measured by MTT assay) of the corresponding aerosols (20% non-cytotoxic and 54% cytotoxic). Creamy/buttery flavoured aerosols were more cytotoxic compared with other flavour groups. Aerosols generated from VG-based refill fluids were cytotoxic (91%) and produced greater cytotoxicity when compared with PG and PG/VG-based products. hESC were generally more sensitive to aerosols than hPFs and A549
Lee et al., 2018, US (258)	To determine the effect of VP metabolites on the susceptibility to mutations and	Human lung (BEAS-2B) and bladder (UROtsa) epithelial cells	Different concentrations of nicotine (BEAS-2B: 0-200 μM; UROtsa: 0-5 μM), and NNK (BEAS-2B: 0-1,000 μM; UROtsa: 0-400 μM) for	Both nicotine and NNK induced DNA adducts (γ-OH-PdG adducts, and O6-medG), reduced DNA-repair activity and the level of repair proteins XPC and

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	tumorigenic transformation of cultured human cells		1h	hOGG1/2, as well as enhanced mutational susceptibility (2-4 fold) and cell transformation in lung and bladder epithelial cells.
Bahmed et al., 2019, US (306)	To examine the cytoprotective effect of DJ-1 against VP-induced human primary alveolar type II cell injury	Human alveolar type II (ATII) cells without or with DJ-1 knockdown	Aerosol from nicotine-free or 24mg/ml nicotine fluid, 2s/puff every 30s, for 1h, analysed 24h post-exposure	VP exposure increased IL-8 levels and induced DNA damage and apoptosis. DJ-1 knockdown in ATII cells sensitized cells to mitochondrial dysfunction (high mitochondrial superoxide production), decreased mitochondrial membrane potential, and calcium elevation.
Chatterjee et al., 2019, US (69)	To evaluate the acute response to nnVP aerosol in terms of oxidative stress and indices of endothelial activation in vitro	Human pulmonary microvascular endothelial cells (hPMVEC)	HPMVEC treated with cell media supplemented with 15% of serum collected from 10 subjects before (-30min) and after (30-360min) exposure to VP VP aerosol using E-puffer, 3.7V, 2.7Ω, 70%PG/30% VG without nicotine, 2s/puff, 16-17 inhalations (equivalent to 1 cig)	VP aerosol inhalation led to a transient increase in markers of oxidative stress (ROS production) and inflammation (soluble intercellular adhesion molecule, ICAM-1).
Muthumalage et al., 2019, US (307)	To examine whether flavourings used in VP cause oxidative	Human bronchial epithelial cells (16-HBE and BEAS-2B cell lines)	JUUL pod flavours: Fruit Medley, Virginia Tobacco, Cool Mint, Crème Brulee,	VP exposures induced acellular ROS (6 JUUL pod flavours) and mitochondrial superoxide

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	stress, barrier dysfunction and infammatory responses in lung epithelial cells and monocytes	Monocytes (U937 cell line)	Cool Cucumber, Mango, and Classic Menthol) with 5% nicotine. Other pod flavours: 'Just Mango' (strawberry and coconut), and Café Latte with 6% nicotine For ROS assessment: 3s puff with 17s interval, 3puffs per min, 5-15puffs bubbled through the fluorescent dye For other experiments: aerosolised, 3s puff with 17s intervals, 66 puffs during 22minutes, three sessions with 12h intervals	production (only 4 flavours tested) in 16-HBE. Differential inflammatory responses observed in all VP-exposed cell lines depending on the flavour (increased IL-8 or PGE2). JUUL pod aerosols (Crème Brulee and Cool Cucumber) caused epithelial barrier dysfunction in 16-HBE cells as shown by decreased normalized resistance and voltage in the epithelial membrane. Moreover, Cool Cucumber, Classic Menthol, Just Mango and Café Latte flavoured pods also showed DNA damage (assessed by Comet assay) upon exposure in monocytes.
Rowell et al., 2019, US (308)	To investigate whether exposure to commercially available e-liquids can alter cytosolic Ca2+ levels, an important second	Primary human bronchial epithelial cells (hBEC), Calu-3 airway cell line and HEK-293T cells	100 flavoured e-liquids were diluted and used in the fluo 4 Ca2+ screen Diluted 55% PG/ 45% VG alone or Banana Pudding e-liquid without or with	42 of 100 flavoured e-liquids elevated cytosolic Ca2+ levels in Calu-3 cells. Banana Pudding (BP) aerosol elicited acute increases in cytoplasmic Ca2+ in Calu-3 cells.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	messenger that can regulate cell growth/survival		12mg/mL nicotine (3h or 24h) and corresponding aerosols (70ml/3s/puff, 0-25 puffs)	Short exposure to Banana Pudding e-liquid caused phospholipase C activation, endoplasmic reticulum (ER) Ca2+ release, store-operated Ca2+ entry (SOCE), and protein kinase C (PKCα) phosphorylation, while longer exposure depleted ER Ca2+ stores and inhibited SOCE in multiple cell lines
Lamb et al., 2020, US (309)	To determine mitochondrial respiration and electron transport chain protein levels after exposing lung epithelial cells to JUUL pod-based aerosols	Lung epithelial cells (BEAS-2B)	Pod-based Menthol or Virginia Tobacco flavoured 5% nicotine aerosols, 55ml/puff, 3puffs/min, 3 x 66puffs (22min) with 12h intervals Analysed immediately and 24h post-exposure	Menthol pod exposure resulted in an alteration in mitochondrial respiration with an immediate increase in proton leak, decrease in coupling efficiency, and alteration of electron transport chain (ETC) protein levels, while 24h post-exposure resulted in reduced basal respiration, maximal respiration, and spare capacity. Tobacco pod exposure had no effect on mitochondrial respiration, but ETC subunits were increased immediately post-final exposure.
Pushalkar et al., 2020, US (310)	To evaluate the influence of VP	The squamous cell carcinoma Fadu cell	Aerosol from disposable VP with 24mg/mL nicotine,	In vitro infection model of premalignant Leuk-1 and

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	aerosols on infection efficiency of oral pathogens in pre-cancerous and cancer cell lines	line and human oral mucosal epithelial (Leuk-1) cell line	2puffs/min for 40 min followed by infection with P. gingivalis and F. nucleatum for 2 h	malignant cell lines exposed to VP aerosol and challenged by bacteria infection resulted in elevated inflammatory response as evidenced by increased expression of cytokine mRNA, such as TNF-α, IL-8, IFN-γ, IL-1β, and IL-6, compared to those exposed to air co-infected with the same bacteria. Protein levels of IL-8 were increased in response to VP aerosol.
Jarell et al., 2021, US (311)	To examine the impacts of first-hand and second-hand exposure levels to maltol-flavoured VP vapours on lung metabolism	Human bronchial epithelia cells (BEAS-2B)	Aerosols from 30%PG/70%VG with or without nicotine or maltol-flavoured PG/VG with nicotine (3.9 mM maltol and 100 μM nicotine for first-hand exposure, 3.9 μM maltol and 100 nM nicotine for second-hand exposure) 70ml/3.3s puff, 1puff/min, for 1h	Both first- and second-hand exposure to maltol-flavoured VP aerosols affected lung airway epithelial cell metabolism and disrupted metabolism of amino acids, as well as other pathways. Oxidative stress was present with VP exposure, as indicted by glutathione and cystine concentrations and protein S-glutathionylation, but not affected by maltol.
Khalil et al., 2021, Lebanon (312)	To determine the acute cytotoxic, genotoxic and cell damage impacts associated with VP consumption using	Human pulmonary cells (A549)	VP aerosols from citrus, ‘double apple’ and ‘Italian’ flavours without nicotine or ‘Rich tobacco’ flavour with 1.6mg/mL nicotine, 60ml/3s/puff every 20s, 0-	A dose-dependent decrease in viability of A549 cells exposed to VP aerosols with a statistically significant decrease in all treatments recorded at 30-60 puffs (MTS assay, p<.05) with the

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	a novel dynamic exposure methodology		30 puffs at different wattage (0-60W). Use two types of rechargeable VP.	nicotine containing VP aerosol being the least toxic. VP aerosols displayed genotoxic potential (citrus, apple and tobacco-flavoured aerosols) and apoptotic induction (all flavours) in exposed cells after 30 puffs at 40W. Nicotine containing VP aerosol displayed the most significant late apoptosis combined with loss of cell membrane integrity.
Ji et al., 2019, US (313)	To examine if VP exposure impacts the gene pathways of normal human oral keratinocytes, particularly the unfolded protein response (UPR) pathway	Normal human oral keratinocytes (NHOK)	29.3% PG/68.3% VG with 2.4 mg/l nicotine, aerosols were impinged into the culture media during 15min, that was incubated with cells for 4h	In response to VP aerosol treatment, a number of functional pathways were found to be activated in NHOK (by DNA microarray analysis), including unfolded protein response, protein ubiquitination, cell cycle regulation, oxidative stress response, NF-κB signalling, IL-6, -8, and -10 signalling, TGF-β signalling, HGF signalling and EMT regulation. Importantly, VP exposures up-regulated the UPR pathway genes, C/EBP homologous protein (CHOP, fold change

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
				43.1), activating transcription factor 4 (ATF4), X box binding protein 1 (XBP1), and inositol-requiring enzyme 1 alpha (IRE1α), while immunoglobulin heavy-chain binding protein (BIP) and PRKR-like ER kinase (PERK) expression was also increased, but not significantly. These changes were confirmed at the protein level.
Song et al., 2020, Korea (314)	To examine the effects of VP aerosol exposure with or without nicotine on mucin production by human airway epithelial cells	Human airway epithelial cells (NCI-H292) Human primary nasal epithelial cells	Aerosol from 50%PG/50%VG without or with 24 mg/mL nicotine, vapour was bubbled through 20ml media 20 or 40 times, 4s puff every 60s	In both NCI-H292 cells and human nasal epithelial cells, VP exposure stimulated MUC5AC, but not MUC5B, expression regardless of nicotine content via activation of the mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling pathways. No cytotoxic activity was observed after VP exposure.
Miyashita et al., 2018, UK (315)	To determine the effect of VP aerosol on platelet-activating factor receptor (PAFR)-dependent pneumococcal	Human alveolar type II epithelial cell line (A549) Human bronchial epithelial cell line (BEAS-2B)	VP aerosol extract collected (25puffs over 5min) using tobacco flavoured fluid without or with 24mg/mL nicotine, 0%-5% (0-0.4 mg/mL nicotine)	VP aerosol, regardless of nicotine, increased pneumococcal adhesion to all airway cells. VP-stimulated adhesion was completely attenuated by the PAFR blocker CV3988.

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	adhesion to airway cells in vitro	Human primary bronchial epithelial cells (HBEpCs) Human primary nasal epithelial cells (HPNEpCs)		
Others				
Studies reporting adverse effects of VP and TC exposure				
Lee et al., 2019, US (316)	To investigate the effects of flavoured e-liquids and serum isolated from VP users on endothelial health and endothelial cell dependent macrophage activation	Human induced pluripotent stem cell-derived endothelial cells (iPSC-EC)	Serum from VP users (RY4-flavoured 16mg/ml nicotine, 2s puff every 30sec for 10min) and TC users (Marlboro, 2puffs/min, for 10min or 1cig), obtained 0 ,1 and 3h post-exposure 6 flavoured e-liquids with 50%PG/50%VG, 80%PG/20%VG and 100%VG in 0, 6 and 18mg/mL nicotine concentrations, 0-1% dose for 48h	Treatment with serum from VP and TC users increased ROS associated with endothelial dysfunction, as shown by altered tube formation in iPSC-EC. Also, there was increased expression of inflammatory cytokines in serum of VP and TC users. Treatment with flavoured e-liquids exacerbated endothelial dysfunction as indicated by increased cytotoxicity (especially with cinnamon-flavour), decreased cell viability, increased reactive oxygen species (ROS) levels, caspase 3/7 activity, and low-density lipoprotein uptake, activation of oxidative stress-related pathway, and impaired

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
				tube formation and migration.
Studies reporting adverse effects of VP exposure				
Zahedi, 2019, US (317)	To characterize the effects of VP liquids and their aerosols on stem cell mitochondria and to identify the ingredient in VP products that activates stress-induced mitochondrial hyperfusion (SIMH)	Mouse neural stem cells	Cell treated with menthol and tobacco flavoured e-liquids with nicotine (0.3%, 0.5%, and 1% dilutions) or corresponding aerosols captured in cell medium (4.3puff every min, 1.8, 3 and 6 total-puff-equivalents) for 4h and 24h	Exposure of stem cells to e-liquids and aerosols produced a stress response that led to interruption of autophagic flux without mitophagy and SIMH, which was accompanied by alterations in mitochondrial morphology and dynamics, increased mitochondrial superoxide levels and protein oxidation, altered mitochondrial membrane potential, induced aggregation of mitochondrial nucleoids and mtDNA damage, and accumulation of calcium. Importantly, nicotine alone can induce the changes observed with VP aerosols
Abouassali et al., 2021, US (318)	To assess the cardiac electrophysiological toxicity of flavoured VP, and to test whether vaping can result in cardiac electrophysiological instability and inducible	Mouse atrial cardiomyocytes (HL-1) Human induced pluripotent stem cell (hiPSC)-derived cardiomyocyte culture Human embryonic kidney (HEK 293) cells	VP aerosol extract from 30%PG/70%VG alone, with 6mg/ml nicotine or with nicotine and flavourings (Vanilla custard, Hawaiian POG (passion fruit, orange, and guava) and Apple Jax (milky cinnamon apple cereal)), 110ml/4.7s puff, 15 puffs in 10ml of media,	Vanillin and cinnamaldehyde (Apple Jax) flavoured VP aerosol extracts were more toxic in HL-1 cardiomyocytes than fruit-flavoured extracts (assessed by annexin V staining). Exposure of hiPSC-derived cardiomyocytes to cinnamaldehyde or vanillin-flavoured VP extracts affected the beating frequency and

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	arrhythmogenesis	cotransfected with wild-type the human ether-à-go-go-related gene (hERG) and green fluorescent protein	tested concentrations were 0.075, 0.15, 0.375, and 0.75 puffs/mL, cultured for 24 and 48h	prolonged the field potential duration of these cells more than fruit-flavoured extracts. Additionally, vanillin aldehyde-flavoured VP extract reduced the hERG-encoded potassium current in transfected HEK293 cells.
Gene regulation (not in vitro studies)				
Studies reporting adverse effects of VP and TC exposure				
Corbett et al., 2019, US (126)	To identify the impact of VP use on airway gene expression	Bronchial airway epithelial cells	Cells collected from current VP users 6 days/week for ≥1 month (n=15), current TC smokers with ≥5cig/day (n=9) and former TC smokers, tobacco abstinent for at least 3 months (n=21)	The pattern of gene expression in VP users was much more similar to former smokers than to active TC smokers. Analysis revealed 3,165 differentially expressed genes associated with any smoking status (p<.05) that included 468 genes whose expression was dependent on VP use (p<.05). 79 of these genes were up- or down-regulated concordantly among VP users and TC smokers.
Tommasi et al., 2019, US (319)	To investigate the regulation of genes and associated molecular pathways, genome-wide, in oral cells of VP	Oral epithelial cells	Cells obtained from VP users (≥3times/week, ≥6months), TC smokers (≥3 times/week, ≥1 year), and non-smokers (n = 42, 24, and 27, respectively)	RNA-sequencing analysis showed deregulation of critically important genes and associated molecular pathways in both VP users and TC smokers, although smokers had 50% more

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	users and TC smokers as compared to non-smokers			differentially expressed transcripts than vapers (1726 vs. 1152). The majority of these genes were associated with cancer-related pathways and functions in both groups (~62% in VP vs. 79% in TC). While high proportion of deregulated transcripts in smokers were from protein-coding genes (79% vs. 53% in vapers), nearly 28% of the aberrantly expressed transcripts in vapers (vs. 8% in smokers) belonged to regulatory non-coding RNAs. The most affected pathways were found to be “Wnt/Ca+ pathway” in vapers and the “integrin signaling pathway” in smokers.
Lee et al., 2020, US (320)	To understand the potential relationship between vaping/smoking and the dysregulation of key genes and	Bronchial epithelial cells (H292) at the ALI	Three independent RNA expression datasets from smokers and vapers were obtained from GSE138326 (n =15 VP, n=15 non-smokers): VP group used nicotine-free	Both TC and flavoured/nicotine-containing VP use led to upregulation of pro-inflammatory cytokines, such as CCL20 and CXCL8 in smokers, and CCL5 and CCR1 in flavour/nicotine-containing VP users, and

Authors	Study aim	Cells or tissue type	Test agent and exposure	Main outcomes (vs. control unless otherwise stated)
	pathways related to COVID-19.		50%PG/50%VG without flavours, 2 times/day, 20 puffs/session for 4 weeks And GSE112073 (n=15 VP, n=21 non-smokers): VP group used nicotine-containing VP of any flavour, 6 days/week for at least 1 month. Data on TC smokers was obtained from 49 lung squamous cell carcinoma (LUSC) patients	inflammasome-related genes, including CXCL1, CXCL2, NOD2, and ASC in both groups. TC smoking, but not VP use, upregulated ACE2, the receptor for the SARS-CoV-2 viral entry. Flavouring- and nicotine-free VP exposure did not lead to cytokine dysregulation and inflammasome activation.

Notes: ALB – albumin; ALI – air-liquid interface; ATF4 – activating transcription factor 4; ATII – alveolar type II; ATP – adenosine triphosphate; BIP – immunoglobulin heavy-chain binding protein; CBD – cannabidiol; CFTR – cystic fibrosis transmembrane conductance regulator; CXCL1/CXCL2 – chemokine ligand 1 or 2; CYP – cytochrome P450; EMT – epithelial–mesenchymal transition; GLUT-1 – glucose transporter 1; hBEC – human bronchial epithelial cells; hERG – human ether-à-go-go-related gene; hESC – human embryonic stem cells; HGF – hepatocyte growth factor; hiPSC – human induced pluripotent stem cell; HMOX1 – heme oxygenase 1; hPF – human pulmonary fibroblasts; hPMVEC – human pulmonary microvascular endothelial cells; HTP – heated tobacco product; ILs – interleukins; IRE1α – inositol-requiring enzyme 1 alpha; LDH – lactate dehydrogenase; MAPK – mitogen-activated protein kinase; MCP1 – Monocyte chemoattractant protein-1; MMP12 – matrix metallopeptidase 12; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; MTV – mucous transport velocity; nAChR – nicotinic acetylcholine receptor; NHOK – normal human oral keratinocytes; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; NNK – 4-(methylnitrosamino)-1- (3-pyridyl)-1-butanone; NQO1 – NAD(P)H Quinone Dehydrogenase 1; nnVP – non-nicotine vaping product; OS – oxidative stress; PAFR – platelet-activating factor receptor; PERK – PRKR-like ER kinase; PG – propylene glycol; ROS – reactive oxygen species; SIMH – stress-induced mitochondrial hyperfusion; TC – tobacco cigarette; TIMP1 – tissue inhibitor

of metalloproteinase-1; TNF – tumor necrosis factor; TW - tobacco waterpipe; UPR – unfolded protein response; VEGFA – vascular endothelial growth factor; : VG – vegetable glycerine; VP – vaping product; XBP1 – X box binding protein 1.

Appendix 8. Table 8: animals and cell studies funding sources as reported in publications

Authors	Funding
Studies that disclosed funding from tobacco industry and/or VP manufacturers	
Banerjee et al., 2017, UK (278)	All of the authors were employed by British American Tobacco (Investments) Ltd, and the study was funded by British American Tobacco (Investments) Ltd. Elements of this work were conducted at Fios Genomics Ltd. as part of a commercial contract.
Fields et al., 2017, US (279)	RAI Services Company bears stewardship responsibility for each of RAI tobacco manufacturing operating companies, namely R.J. Reynolds Tobacco Company (RJRT), American Snuff Co., LLC (ASC), and Santa Fe Natural Tobacco Company, Inc. (SFNTC).
Adamson et al., 2018, UK (280)	All authors were full time employees of British American Tobacco (BAT). This study was fully funded by BAT. The authors have no competing interests.
Glynos et al., 2018, Greece (213)	This study was funded in part by a grant by Nobacco and Alterego, vendors of e-cigarettes (to A. Papapetropoulos).
Czekala et al., 2019, UK (282)	This work was funded and supported by Fontem Ventures B.V. Imperial Brands Group PLC is the parent company of Fontem Ventures B.V., the manufacturer of the commercial e-liquid used in this study.
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