TOX/2019/39

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 13: User exposure.

Background

1. The COT is reviewing the potential toxicity of electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (collectively abbreviated to E(N)NDS). As part of this review, a paper on characterisation of the aerosol particle fraction (TOX/2017/49) was discussed at the December 2017 COT meeting, and papers summarising evaluations of metals (TOX/2018/15) and some other constituents (TOX/2018/16) in E(N)NDS products were presented at the March 2018 COT meeting. These 3 discussion papers focussed on constituents sampled in E(N)NDS liquids or in 'firsthand' E(N)NDS aerosols produced from the E(N)NDS product (without having been inhaled by a user), with relevance to evaluating potential levels of exposure to the E(N)NDS user. The aim of the present discussion paper is to estimate the levels of some of these constituents to which users of E(N)NDS may be exposed. In addition, studies that have measured biomarkers of exposure to tobacco-related toxicants in E(N)NDS users are reviewed. Flavouring compounds are not included as they are being addressed separately.

Introduction

2. E(N)NDS are battery-powered devices containing a liquid (E(N)NDS liquid or 'e-liquid'). The E(N)NDS liquid is heated on use to produce an aerosol that is inhaled by the user ('puffing', 'vaping'). E(N)NDS were first introduced commercially in China in 2004 and subsequently in the EU (2005) and USA (2007) as nicotine-delivery devices. The main constituent parts of an E(N)NDS device are a mouthpiece, cartridge (tank) containing E(N)NDS liquid, a heating element/atomizer, a microprocessor, a battery, and sometimes an LED light. Commercially available devices are sometimes categorised as first, second, or third generation. First-generation devices look like conventional cigarettes (CC) and thus are termed 'cigalikes'. Initial models comprised three principal parts; a lithium-ion battery, a cartridge and an atomizer. However, more recent models mostly consist of a battery connected to a 'cartomizer' (cartridge/atomizer combined), which may be replaceable, but is not refillable. Second-generation E(N)NDS are larger and have less resemblance to tobacco cigarettes. They often resemble pens or laser pointers (hence the name, 'vape pens'). They have a high-capacity rechargeable lithium-ion battery and a refillable atomizer (sometimes referred to as a 'clearomizer'). Third-

generation models ('advanced personal vapers', 'mods') are also refillable, have very-high-capacity lithium-ion batteries and are highly customisable (different coil options, power settings, tank sizes). In addition, highly advanced 'fourth generation' E(N)NDS (innovative regulated mods) are now being described.

3. Constituents that have been identified in E(N)NDS liquids and/or aerosols include propylene glycol (PG), glycerol (glycerin(e), vegetable glycerine (VG)), water, nicotine, carbonyls, volatile organic compound (VOCs), tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), metals, ethanol, ethylene glycol, di-ethylene glycol, flavouring compounds, flavour enhancers, sweeteners, and phenolics.

Information from previous COT discussion papers on E(N)NDS liquid and aerosol constituents

4. Data on reported levels of some of constituents in E(N)NDS liquids and aerosols were summarised in discussion papers TOX/2018/15 and TOX/2018/16, presented at the February 2018 COT meeting. Prior to this, TOX/2017/49, discussed at the December 2017 COT meeting, summarised studies that investigated the particulate matter in E(N)NDS aerosols. Narrative text from these previous COT discussion papers is summarised in paragraphs 5–11, below.

5. The principal components (often in the range of 90-95% of the mass) of most E(N)NDS liquids are the solvents, PG and glycerol, which can be present in ratios ranging from 0:100 to 100:0 (Pellegrino et al. 2012, Hahn et al. 2014, Schober et al. 2014, Tayyarah and Long 2014, Geiss et al. 2015, Han et al. 2016, Sleiman et al. 2016, Etter and Bugey 2017, Peace et al. 2017). Other common additives are water, nicotine, and flavouring compounds.

6. Nicotine concentrations stated on product labels are generally in the range of up to 20 mg/mL, although products with higher nicotine concentrations may be available in some countries. In the UK, the Tobacco and Related Products Regulations 2016 states that "nicotine-containing liquid which is presented for retail sale in an electronic cigarette or refill container must not contain nicotine in excess of 20 milligrams per millilitre" (Part 6, section 36(4)). Several investigations have found that nicotine concentrations do not always correlate well with levels stated on the label (Hadwiger et al. 2010, Trehy et al. 2011, Davis et al. 2015, Han et al. 2016, Peace et al. 2016, Sleiman et al. 2016, Cheah et al. 2014).

7. Some studies have reported the presence of contaminants and impurities in E(N)NDS liquids, often at low or trace levels, for example, ethylene glycol. The nicotine in E(N)NDS liquids is usually derived from tobacco plants and may contain contaminants, including minor tobacco alkaloids (reported levels at approximately 1-2% of the nicotine content in one study) (Etter, Zather and Svensson 2013, Lisko et al. 2015, Flora et al. 2016, Han et al. 2016, Sleiman et al. 2016) and TSNAs (maximum values reported, approximately 60 ng/mL N-nitrosonornicotine (NNN), 10 ng/mL 4-(methylnitrosamino)-1–(3-pyridyl)-1-butanone (NNK), 11 ng/mL N-

nitrosoanabasine (NAB), 62 ng/mL N′-nitrosoanatabine (NAT)) (Kim and Shin 2013, Farsalinos et al. 2015, Han et al. 2016). One report described the presence of low levels of diethyl phthalate (DEP) and diethylhexyl phthalate (DEHP) in some E(N)NDS liquids (Oh and Shin 2015). Ethanol, which may be present as a solvent in flavouring compounds has also been detected (Sleiman et al. 2016, Poklis, Wolf and Peace 2017). Analysis of some E(N)NDS liquids has identified other active compounds, for example a weight-loss drug (Hadwiger et al. 2010) and a synthetic cannabinoid (Peace et al. 2017, Poklis et al. 2017). Several studies have reported E(N)NDS liquid samples that are not true-to-label.

8. Aerosol is produced by heating of the E(N)NDS liquid within the E(N)NDS device. Glycerol and PG have different physical properties (for example, glycerol has a higher boiling point) and this may affect factors such as the temperature at which the aerosol is produced and the size-distribution properties of the particles, which will affect the region of deposition in the airway. Studies of E(N)NDS aerosol particulate matter (see TOX/2017/49 for bibliography) suggest that it comprises submicronic particles with a similar size distribution to CC smoke, and also nanoparticles. Particle number concentrations (PNC) in undiluted mainstream aerosol are generally reported in the range of 10^9 particles/cm^3. The relative proportions of submicronic and nano particles are difficult to estimate due to experimental limitations, including substantial evaporation of larger particles at high dilution ratios and limited capability of spectral transmission methods to detect nanoparticles.

9. Solid particles, such as metal nanoparticles, may also be present in E(N)NDS aerosols. E(N)NDS contain metal components in the structure of the device, which may include resistive wire heating filaments, wire couplings, solder joints, and silver coatings. In most cases, the filament is composed of nickel and chromium, although other metals are present in some devices. Thick wire usually consists of copper (but sometimes copper/nickel) coated with silver (sometimes tin). Joints may be brass (copper/zinc) or solder, which is mostly tin, but in some cases has also been found to contain lead. Insulating sheaths generally contain silicon, calcium, aluminium, and magnesium, and the wick is usually made of fibreglass containing silicon. Overall, the concentrations of metals in E(N)NDS aerosols have been observed to vary widely (by several orders of magnitude) both between and within brands. Reasons for this may include structural aspects of the E(N)NDS device (including manufacturing inconsistencies, variations in wire resistance and battery voltage, and E(N)NDS liquid delivery rate), puffing protocols, variation in E(N)NDS liquid components, and changes occurring with use and storage of products. Studies published to date have mostly investigated first- or second-generation E(N)NDS devices. There is variability in the findings between studies. A principal source of this variability is likely to be the methods used to extrapolate from the amount of metals captured on the filter to the amount of metals present in the aerosol, with potential for both over- and under-estimation depending on how capture efficiency is corrected for. Additionally, in many cases the reported measurements are around the limit of

detection (LOD)/ limit of quantitation (LOQ) for the detection method used, though the values of the LOD/LOQ are not always provided.

10. Thermal decomposition of E(N)NDS liquids during aerosol production may lead to the production of degradation products, for example carbonyl compounds such as formaldehyde, acetaldehyde, and acrolein, with levels reported ranging widely (from a few ng to > 20 µg per puff) (Uchiyama et al. 2013, Goniewicz et al. 2014, Czogala et al. 2014, Hutzler et al. 2014, Kosmider et al. 2014, Geiss et al. 2015, Herrington and Myers 2015). The extent to which thermal breakdown occurs is likely to be related to user behaviour (puffing parameters) and the operating characteristics of the E(N)NDS device, such as battery output and heating-coil resistance, which affect the temperature attained (Jensen et al. 2015, Sleiman et al. 2016, Uchiyama et al. 2016, Ogunwale et al. 2017). For example, formaldehyde emission levels in the range of approximately 80-100 µg/puff have been reported using variable-voltage devices on very high settings (in the range of 5.0 V or 15 W) (Ogunwale et al. 2017, Sleiman et al. 2016), although emission levels reported from use at lower voltage/power settings are generally much lower than this. It has also been suggested that standard methods of analysis underestimate the levels of formaldehyde produced (Salamanca et al. 2017). This is currently an area of active investigation and debate, with some commentators asserting that carbonyl production only occurs during 'dry puffing' (i.e. in the absence of E(N)NDS liquid), which would be avoided by E(N)NDS users due to the disagreeable experience (Farsalinos et al. 2017, Farsalinos et al. 2018).

11. The method by which the E(N)NDS liquid is applied to the heating coil has been reported to affect levels of degradation products in the aerosol. In most E(N)NDS apparatus, liquid is drawn to the coil through a wick. However, some newer devices allow 'direct dripping' of liquid onto the heating element, which appears to be associated with substantially increased levels of carbonyl emissions (Talih et al. 2016). Reported proportions of nicotine emitted from E(N)NDS liquids to aerosols on puffing vary, and this also likely depends on a combination of device characteristics, puffing behaviour, and the overall composition of the E(N)NDS liquid (Talih et al. 2016). Some studies have reported the detection of VOCs, e.g. benzene or toluene, and PAHs in E(N)NDS emissions, although generally at very low levels (Goniewicz et al. 2014, Tayyarah and Long 2014, Margham et al. 2016, Lee et al. 2017).

Literature source and summary of data by type of chemical or species analysed

12. Except for the section on 'Biomarkers of Exposure to E(N)NDS' (see paragraphs 28–68), data included in this present discussion paper are taken from the publications cited in the previous COT discussion papers, TOX/2017/49, TOX/2018/15, and TOX/2018/16.

13. The publications cited in TOX/2017/49, TOX/2018/15, and TOX/2018/16, which reported data on levels of particulate and/or gas-phase components of E(N)NDS aerosols, are summarised in Table 1, below.

Table 1. Studies listed in COT discussion papers TOX/2017/49, TOX/2018/15, and TOX/2018/16 that evaluated levels of particulate- and/or gas-phase components detected in E(N)NDS aerosols.

Constituent(s)	Studies	Constituent(s)	Studies
Particulate matter	Ingebrethsen et al. (2012)[1] Alderman et al. (2014)[2] Fuoco et al. (2014) Blair et al. (2015) Mikheev et al. (2016) Zhao et al. (2016) Baassiri et al (2017) Belka et al. (2017) Kim et al. (2017) Lee et al. (2017)	Carbonyls	Uchiyama et al. (2013) Goniewicz et al (2014) Hutzler et al. (2014) Kosmider et al. (2014) Tayyarah and Long (2014) Geiss et al. (2015) Herrington and Myers (2015) Jensen et al. (2015) Laugesen (2015) Flora et al. (2016) Margham et al. (2016) Sleiman et al. (2016) Talih et al. (2016) Uchiyama et al. (2016) Farsalinos et al. (2017)[3] Ogunwale et al. (2017) Salamanca et al. (2017) Farsalinos et al. (2018)[4]

[1] Declaration of Interest: 'The work presented here was funded by R.J. Reynolds Tobacco Company.'
[2] Corresponding author address: R.J.Reynolds Tobacco Co.
[3] Conflict of interest statement: 'In the past 36 months, 2 studies by KF were performed using unrestricted funds from the non-profit association AEMSA and 1 study by the non-profit association Tennessee Smoke-Free Association.' Funding: 'No funding was provided for the study.'
[4] Conflicts of interest: 'In the past 3 years, KF has published 2 studies funded by the nonprofit association AEMSA and 1 study funded by the non-profit association Tennessee Smoke-Free Association. KK, AP, AS, KP and GG have no conflict of interest to report. Enthalpy Analytical is a for-profit CRO involved in analytical testing of tobacco and e-cigarette products.' Funding: 'No funding was provided for the study.'

Constituent(s)	Studies	Constituent(s)	Studies
PG and/or glycerine	Pellegrino et al. (2012) Kienhuis et al. (2015)[5] Margham et al. (2016)[6]	TSNAs	Trehy et al. (2011) Goniewicz et al. (2014)[7] Tayyarah and Long (2014) Farsalinos et al. (2015)[8] Flora et al. (2016) Margham et al. (2016)

[5] This study was not included in TOX/2018/16 but has been added here as it was noted in other COT discussion papers on E(N)NDS and was considered to be of relevance here.

[6] Funding: 'The research described in this article was funded by British American Tobacco Investments Ltd.'
Notes: 'The authors declare the following competing financial interest(s): All authors are employees of British American Tobacco. British American Tobacco is the sole source of funding and sponsor of this project.'

[7] Competing Interest: 'Dr. Goniewicz received research funding from Pfizer, manufacturer of stop smoking medication and is currently funded by the UK Center for Tobacco Control Studies, UK Public Health Centre of Excellence (UKCTCS). UKCTCS receives it funding from the Economic and Social Research Council (ESRC), British Heart Foundation (BHF), Cancer Research UK, National Institute for Health Research (NIHR), and Medical Research Council (MRC). Dr. Benowitz is a consultant for several companies that market smoking cessation medications and has been a paid expert in litigation against tobacco companies. The other authors declare they have no actual or potential competing financial interests.'

[8] Conflicts of Interest: 'Some of the studies by Konstantinos E. Farsalinos and Vassilis Voudris were performed using funds provided to the institution by e-cigarette companies. Gene Gillman and Konstantinos Poulas have no conflict of interest to report.'

Constituent(s)	Studies	Constituent(s)	Studies
Nicotine and tobacco alkaloids	Trehy et al. (2011) Pellegrino et al. (2012) Czogala et al. (2014)[9] Tayyarah and Long (2014)[10] Laugesen 2015)[11] Flora et al. (2016)[12] Margham et al. (2016) Sleiman et al. (2016) Talih et al. (2016) Baassiri et al. (2017) Lee et al. (2017)	Metals	Williams et al. (2013) Goniewicz et al. (2014) Saffari et al. (2014) Schober et al. (2014) Tayyarah and Long (2014) Farsalinos et al. (2015)[13] Lerner et al. (2015) O'Connell et al. (2015)[14] Williams et al. (2015) Margham et al. (2016) Mikheev et al. (2016) Kim et al. (2017) Lee et al. (2017) Liu et al. (2017)[15] Oldham et al. (2017)[16] Palazzolo et al. (2017) Williams et al. (2017) Olmedo et al. (2018)

[9] Declaration of Interests: 'MLG received research funding from Pfizer, manufacturer of stop smoking medication, and was funded by the UK Centre for Tobacco Control Studies (UKCTCS) during the study. AS received research funds and travel expenses from Chic Group Ltd., manufacturer of electronic cigarettes in Poland. Other authors declare no conflicts of interest.'

[10] Conflicts of interest: 'The company for which the study authors work and the companies that manufacture the e-cigarettes tested for this study are owned by the same parent company.'

[11] Author information: 'Murray Laugesen, Public Health Medicine Specialist, and owner of Health New Zealand
Ltd (a nicotine and tobacco research and policy consultancy), Christchurch.'
Competing interests: 'Agencies which sold EasyPuff, Elusion, Greensmoke, and KiwiCig contributed to expenses of testing, as had Ruyan for 2008 samples. These contributions did not influence the design or conclusions of this study.'

[12] Corresponding author: Altria Client Services LLC

[13] Conflicts of Interest: 'The authors declare no conflict of interest. No funding or any other support was provided for this study. A small minority of the studies by Konstantinos Farsalinos and Vassilis Voudris were performed using unrestricted funds provided to the institution (Onassis Cardiac Surgery Center) by e-cigarette companies.'

[14] Conflicts of Interest: 'All authors are employees of Imperial Tobacco Group. The work in this manuscript was supported by Imperial Tobacco Group. Imperial Tobacco Group is the parent company of Fontem Ventures B.V.,
the manufacturer of the e-cigarette products used in this study.'

[15] Conflicts of Interest: 'The study was funded by Altria Client Services LLC. The authors, Mohamadi Sarkar, Jianmin Liu, Qiwei Liang, Michael J. Oldham, Ali A. Rostami and Karl A. Wagner are employees of ALCS. I. Gene Gillman, Piyush Patel

Studies in italics either reported a conflict of interest in terms of funding of the study or the study authors are direct employees of a tobacco company.

14. As has been noted in previous discussion papers, studies that have presented data on levels of constituents measured in E(N)NDS aerosols can be difficult to compare due to the lack of standardisation and the wide range of test conditions and methodologies used. These include variations in the products tested (types of E(N)NDS devices and E(N)NDS liquids), aerosol generation parameters, sampling and detection methodologies, LOD/LOQs of the methods used, controls, constituents sampled, and statistical analyses and reporting of data.

15. Taking into account the limitations of the available literature, as far as is possible from the data set, levels of chemicals and/or particulate matter that have been reported in E(N)NDS aerosols are summarised in the sections below, with a view to identifying ranges of levels to which users may be exposed.

Particulate matter

16. A summary of reported levels for PNC and total particulate mass (TPM) is given in Table 2, below.

Table 2. Summary of data reported from studies that have measured PNC and/or TPM in E(N)NDS aerosols.

Study	**E(N)NDS product(s)** **Puff volume (if reported)**	**Particle size range measured (nm)**	**PNC (particles/cm^3), range reported**	**TPM, range reported [conversion for this paper]**
Ingebrethsen, Cole and Alderman (2012)	2 x cartomizer E(N)NDS (1 rechargeable, 1 disposable), liquid constituents not stated 55 cm^3	≤ 1000	Approximately 10^9	0.002–2.5 mg/puff [36–45,454 mg/m^3]

and Rebecca Savioz are paid contractors. The study was conducted on behalf of NuMark LLC., (Richmond, VA, USA) a subsidiary of Altria Group, that produces and markets e-vapor products.'

[16] Conflict of Interest: 'MJO, KAW, JL, AAR, & MS are employees of Altria Client Services, LLC, the sponsor of this work. IGG and JBB are employees of Enthalpy Analytical, Inc., a subcontractor for Inflamax Research Inc., who was contracted by the sponsor to perform this work.'

Study	E(N)NDS product(s) Puff volume (if reported)	Particle size range measured (nm)	PNC (particles/cm^3), range reported	TPM, range reported [conversion for this paper]
Alderman et al. (2014)	Three E(N)NDS products (2 rechargeable cartomizer-type, one disposable), liquids containing PG, glycerol and nicotine 50 mL	56–10,000		1.95–3.07 mg/puff [39,000–61,400 mg/m^3]
Fuoco et al. (2014)	Tank system (rechargeable); Atomiser phantom (rechargeable); Cartom (disposable); liquids of 4 flavours without or with (8-9 mg/mL; 12-18 mg/mL) nicotine	5.6–560	3.26–5.29 x 10^9	
Blair et al. (2015)	E(N)NDS brand not specified, 18 mg nicotine/cartridge in PG, 3.6 V		4.0 x 10^9	
Mikheev et al. (2016)	3 brands of fixed-power, non-refillable 'cigalikes': NJOY King (4.5% nicotine), V2 (0–2.4% nicotine), blu (0–1.6% nicotine); 1 adjustable-power, refillable 'tank-style' Joyetech (0–2.4% nicotine); various flavours	Nanoparticles and submicronic particles	10^7–10^8 per size fraction	

Study	E(N)NDS product(s) Puff volume (if reported)	Particle size range measured (nm)	PNC (particles/cm^3), range reported	TPM, range reported [conversion for this paper]
Zhao et al. (2016)	4 rechargeable E(N)NDS brands (not stated), tobacco flavour, no nicotine; several cartridges tested from each brand	Nanoparticles and submicronic particles	0.58–5.29 x 10^9	
Baassiri et al. (2017)	Vapor-Fi second-generation tank system; liquid batches of analytical-grade PG/glycerol at ratios from 0/100 to 100/0 + 18 mg/mL nicotine, 4.3 W 67 mL (4 s puffs, 16.7 mL/s flow rate)	5.6–560	7.80 x 10^9 (100/0 PG/glycerol) 1.50 x 10^{10} (0/100 PG/glycerol)	19.57 mg/15 puffs (100/0 PG/glycerol) 77.9 mg/15 puffs (0/100 PG/glycerol) [1.30–5.19 mg/puff; 19,461–77,512 mg/m^3]
Belka et al. (2017)	Joyetech refillable, variable wattage (up to 9.6 W); liquid containing 0 or 16 mg/mL nicotine 60 cm^3	Nanoparticles and submicronic particles	4.63–4.81 x 10^9	
Kim et al. (2017)	Custom-built testing device; liquid containing PG/glycerol (1:1, 8:2, 2:8, v/v); 10 mg/mL nicotine 50 mL	500–2000	1903 (mean)	

Study	E(N)NDS product(s) Puff volume (if reported)	Particle size range measured (nm)	PNC (particles/cm^3), range reported	TPM, range reported [conversion for this paper]
Lee et al. (2017)	V2 'cigalike' cartomizer devices (VMR Products): tobacco or menthol flavour; 1.8% nicotine	< 2500 ($PM_{2.5}$); < 100 (nanoparticles)	8000 for $PM_{2.5}$; 5500 for nanoparticles	

The dataset comprises the studies listed in TOX/2017/49.

17. Particle number concentrations were mostly in the range of around 10^9 particles/cm^3, while measured TPM ranged from approximately 0.002 to 5.19 mg/puff, depending on experimental conditions.

18. The highest reported values were:

- PNC: 1.50 x 10^{10} particles/cm^3 (Baassiri et al. 2017)
- TPM: 77.9 mg/15 puffs [equivalent to 5.19 mg/67 cm^3 puff or 77,512 mg/m^3 in aerosol] (Baassiri et al. 2017)

Propylene glycol and glycerol

19. A summary of measured or estimated levels of PG and glycerol in E(N)NDS aerosols is given in Table 3, below.

Table 3. Levels of PG and glycerol in E(N)NDS aerosols.

Study	Test product(s) Puff volume (if reported)	Amount collected from and/or measured in aerosol [conversion for this paper]	
		PG	Glycerol
Pellegrino et al. (2012)	2 x Italian-brand E(N)NDS (liquids contained 66% PG; > 24% glycerol; 0 or 0.25% nicotine	1650–1660 mg/m^3	580–610 mg/m^3
Kienhuis et al. (2015)	4 x disposable shisha pens (liquids contained 54%/46% PG/glycerol; <	0.7 mg/puff [20,000 mg/m^3]	0.6 mg/puff [17,143 mg/m^3]

	1% flavours and other trace components; no nicotine) 35 cm^3		
Margham et al. (2016)	Vype ePen (cartomizer) with 'blended tobacco' e-liquid (liquids contained 25% PG; 48.14% glycerol; 25% water; 1.86% nicotine, <1% flavourings) 55 cm^3	0.709 mg/puff [12,890 mg/m^3]	1.579 mg/puff [28,709 mg/m^3]

Data from studies listed in TOX/2018/16..

20. Few studies reported levels of PG or glycerol measured in E(N)NDS aerosols. The highest levels observed were:

PG: 0.709 mg/55 cm^3 puff [equivalent to 12,890 mg/m^3 in aerosol] (Margham et al. 2016) or 0.7 mg/puff [equivalent to 20,000 mg/m^3 in aerosol] (Kienhuis et al. 2015)

Glycerol: 1.579 mg/55 cm^3 puff [equivalent to 28,709 mg/m^3 in aerosol] (Margham et al. 2016).

Nicotine and related alkaloids

21. A summary of measured levels of nicotine and related alkaloids in E(N)NDS aerosols is given in Table 4, below.

Table 4. Levels of nicotine and related alkaloids measured in E(N)NDS aerosols.

Study	E(N)NDS product(s) and nicotine content Puff volume	Amount collected from aerosol and/or concentration in aerosol (unless otherwise stated) [conversion for this paper]	
		Nicotine	Other alkaloids
Trehy et al. (2011)	4 products purchased via internet; 16 mg/cartridge nicotine(label), 21 mg/cartridge nicotine (measured); 100 mL	50–292 µg/30 puffs [0.67–9.73 µg/puff, 6.7–97.3 mg/m^3]	Anatabine < LOQ (14 µg/30 puffs)
Pellegrino et al. (2012)	Italian-brand E(N)NDS; 0.25% nicotine	6.21 mg/m^3	
Czogala et al. (2014)	3 E(N)NDS products purchased in Poland; 18 mg/cartridge nicotine (label), 11–19 mg/cartridge nicotine (measured); 70 mL	2.51 µg/m^3 (mean) and 0.82–6.23 µg/m^3 (range) in ambient air in a 39m^3 chamber into which 7 x 1.8 s puffs were emitted	
Tayyarah and Long (2014)	2 disposable and 3 rechargeable E(N)NDS; 16–24 mg/unit nicotine (label), 11.7–20.6 mg/unit nicotine (range of mean values for 5 product types) (measured); 55 mL	8–33 µg/puff [145–600 mg/m^3] (range of mean values for 5 product types)	
Laugesen (2015)	14 E(N)NDS products (9 cigalikes, 3 disposables, 2 cartomizers) from China, UK, and USA; 14.5–23 mg/mL nicotine (label), 11.5–27.4 mg/mL nicotine (measured); 70 mL	43 µg/puff [614 mg/m^3] (mean); 18–93 µg/puff [275–1329 mg/m^3] (range)	

Study	E(N)NDS product(s) and nicotine content Puff volume	Amount collected from aerosol and/or concentration in aerosol (unless otherwise stated) [conversion for this paper]	
		Nicotine	Other alkaloids
Flora et al. (2016)	4 E(N)NDS products of 'MarkTen' brand (USA); 1.5% nicotine; 55 mL	29 µg/puff [527 mg/m^3] (average)	
Margham et al. (2016)	Vype ePen (closed modular system with cartomizer, operated at 3.6 V), 'Blended Tobacco' E(N)NDS liquid; 1.86% nicotine; 55 mL	32 µg/puff [582 mg/m^3] (mean)	Myosmine, 0.02737 µg/puff [0.500 mg/m^3] (mean); Cotinine, 0.01084 µg/puff [0.190 mg/m^3] (mean)
Sleiman et al. (2016)	eGO CE4 (single coil); Kangertech AEROTANK mini (dual coil); with Vision Spinner II battery, variable voltage 3.3-4.8 V; 18 mg/mL nicotine (label), 20.4 mg/mL nicotine (measured); 50 mL		Nicotyrine, 31.5 µg/puff [630 mg/m^3] (mean)
Talih et al. (2016) ['direct dripping']	NHALER 510 Atomizer with ego-T battery (3.4 V); PG-based E(N)NDS liquid, with flavour; E(N)NDS use by "direct dripping" of E(N)NDS liquid (dripping every 2, 3, or 4 puffs); 0 or 18 mg/mL nicotine; 152 mL	740–1030 µg/15 puffs [49.3–68.7 µg/puff, or 324–451 mg/m^3] (mean); [620–2950 µg/15 puffs [41.3–197 µg/puff, or 272–1294 mg/m^3] (range)	

Study	E(N)NDS product(s) and nicotine content Puff volume	Amount collected from aerosol and/or concentration in aerosol (unless otherwise stated) [conversion for this paper]	
		Nicotine	Other alkaloids
Baassiri et al. (2017)	Vapor-Fi second-generation tank system; 18 mg/mL nicotine (PG/glycerol mixtures ranging from 0/100 to 100/0) 67 mL (4 s puffs, 16.7 mL/s flow rate)	0.13 mg/15 puffs (0/100 PG/glycerol liquid) 0.58 mg/15 puffs (100/0 PG/glycerol liquid) [0.009–0.039 mg/puff; 129–577 mg/m^3]	
Lee et al. (2017)	V2 'cigalike' cartomizer devices (VMR Products): tobacco flavour, menthol flavour; 1.8% nicotine; (2 puffs/min diluted 1:172 into chamber)	Tobacco-flavoured, 4.35 µg/m^3 (mean); Menthol-flavoured, 2.40 µg/m^3 (mean)	

Data from studies listed in TOX/2018/16.

22. The highest reported values, excluding the study of Talih et al. (2016) that used a direct-dripping E(N)NDS device, were:

Nicotine: 93 µg/70 mL puff [equivalent to 1329 mg/m^3 in aerosol] (product nicotine content, 16 mg/mL label, 15.2 mg/mL measured) (Laugesen 2015)[17]

Nicotyrine: 31.5 µg/50 mL puff [equivalent to 630 mg/m^3 in aerosol] (product nicotine content, 18 mg/mL label, 20.4 mg/mL measured) (Sleiman et al. 2016)

Myosmine: 0.0274 µg/55 mL puff [equivalent to 0.500 mg/m^3 in aerosol] (product nicotine content, 1.86% label) (Margham et al. 2016)

[17] Author information: 'Murray Laugesen, Public Health Medicine Specialist, and owner of Health New Zealand Ltd (a nicotine and tobacco research and policy consultancy), Christchurch.' Competing interests: 'Agencies which sold EasyPuff, Elusion, Greensmoke, and KiwiCig contributed to expenses of testing, as had Ruyan for 2008 samples. These contributions did not influence the design or conclusions of this study.'

Cotinine: 0.0108 µg/55 mL puff [equivalent to 0.190 mg/m^3 in aerosol] (product nicotine content, 1.86% label) (Margham et al. 2016).

TSNAs

23. A summary of measured levels of TSNAs in E(N)NDS aerosols is given in Table 5, below.

Table 5. Levels of TSNAs measured in E(N)NDS aerosols.

Study	E(N)NDS product(s) and nicotine content Puff volume	Level collected from aerosol [conversion for this paper]			
		NNN	NNK	NAT	NAB
Goniewicz et al. (2014)	12 brands (11 from Poland, 1 from UK); 16–18 mg/mL nicotine; 1 nicotine inhaler (reference product) 70 mL	< LOD (not stated) – 4.3 ng/150 puffs [< LOD to 0.029 ng/puff] (mean); (< LOD from nicotine inhaler)	< LOD (not stated) – 28.3 ng/150 puffs [< LOD – 0.189 ng/puff] (mean); (< LOD from nicotine inhaler)		

Study	E(N)NDS product(s) and nicotine content Puff volume	Level collected from aerosol [conversion for this paper]			
		NNN	NNK	NAT	NAB
Tayyarah and Long (2014)	2 disposable and 3 rechargeable E(N)NDS; 16–24 mg/unit nicotine (label), 11.7–20.6 mg/unit nicotine (range of mean values for 5 product types) (measured); Compared with CC (Marlboro Gold Box) (2 results sets presented) 55 mL	E(N)NDS < LOD (0.02 ng/puff); CC 7.93 ng/puff 19.5 ng/puff	E(N)NDS < LOD (0.02 or 0.03 ng/puff); CC 10.12 ng/puff 14.7 ng/puff	E(N)NDS 4 brands < LOD (0.02 or 0.03 ng/puff); Aerosol from 1 brand contained 0.20 ng/puff; CC 9.76 ng/puff 23.5 ng/puff	E(N)NDS < LOD (0.03 or 0.14 ng/puff); CC 1.22 ng/puff 2.67 ng/puff

Study	E(N)NDS product(s) and nicotine content Puff volume	Level collected from aerosol [conversion for this paper]			
		NNN	NNK	NAT	NAB
Farsalinos et al. (2015)	2nd generation eGo tank-style E(N)NDS; 3 tobacco-flavour e-liquids purchased in Greece; 18 mg/mL nicotine (label); 55 mL	< LOD (10_ng/puff)	< LOD (10_ng/puff)	< LOD (10_ng/puff)	< LOD (10_ng/puff)

Study	E(N)NDS product(s) and nicotine content Puff volume	Level collected from aerosol [conversion for this paper]			
		NNN	NNK	NAT	NAB
Flora et al. (2016)	4x MarkTen brand E(N)NDS produced in 2014; 1.5% nicotine; (55 mL, 4 s ?)	< LOQ (40 ng/device)	< LOQ (40 ng/device)		

Study	E(N)NDS product(s) and nicotine content Puff volume	Level collected from aerosol [conversion for this paper]			
		NNN	NNK	NAT	NAB
(Margham et al. 2016)	Vype ePen (cartomizer) with 'blended tobacco' e-liquid (liquids conteined 25% PG; 48.14% glycerol; 25% water; 1.86% nicotine, <1% flavourings); Compared with air/blank and with Kentucky Reference 3R4F CC 55 cm^3	ePen 0.054 ng/puff; Air/blank 0.015 ng/puff; CC 25.0 ng/puff	ePen < LOQ (2.5 ng/100-puff collection); Air/blank < LOQ (2.5 ng/100-puff collection); CC 26.7 ng/puff	ePen < LOQ (3.3 ng/100-puff collection); Air/blank < LOQ (3.3 ng/100-puff collection); CC 25.4 ng/puff	ePen < LOD (0.27 ng/ 100-puff collection); Air/blank < LOD (0.27 ng/100-puff collection); CC 2.85 ng/puff

Data from studies listed in TOX/2018/16. Values reported in square brackets have been calculated using the published data.

24. In the few studies that investigated TSNAs in E(N)NDS aerosols, in general levels of NNN, NNK, NAT, and NAB were below the LOD or LOQ for the analytical method used, or where levels were quantifiable, they were generally very low. In the study of Goniewicz et al. (2014), NNN and NNK were each identified in 9 of 12 samples tested[18], with highest reported individual readings of 0.029 ng/puff for NNN and 0.189 ng/puff for NNK. In the study reported by Margham et al. (2016), NNN was detected at 0.054 ng/puff. In the study of Tayyarah and Long (2014), 1 of the 5 product types tested produced aerosol with a quantifiable content of NAT (0.2 ng/puff). Two studies (Tayyarah & Long 2014, Margham et al. 2016) also provided comparative data on levels of TSNAs in CC smoke, with levels reported in the following ranges: NNN (7.93–25 ng/puff), NNK (10.12–26.7 ng/puff), NAT (9.76–25.4 ng/puff), NAB (1.22–2.85 ng/puff).

Carbonyls

25. A summary of measured levels of carbonyls in E(N)NDS aerosols is given in Table 6, below.

[18] Not the same 9 samples for NNN and NNK

Table 6. Levels of carbonyls measured in E(N)NDS aerosols (data from studies listed in TOX/2018/16). Values reported in square brackets have been calculated using the published data.

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
Uchiyama et al. (2013)	1st generation brands sold in Japan 55 mL	140 µg/10 puffs [14 µg/puff; 255 mg/m^3]	120 µg/10 puffs [12 µg/puff; 218 mg/m^3]	33 µg/10 puffs [3.3 µg/ puff; 60 mg/m^3]	Propanal: 46 µg/10 puffs [4.6 µg/puff; 83 mg/m^3]; Glyoxal: 23 µg/10 puffs [2.3 µg/puff; 42 mg/m^3]; Methylglyoxal: 21 µg/10 puffs [2.1 µg/puff; 36 mg/m^3]
Goniewicz et al. (2014)	12 brands (11 from Poland, 1 from UK) with 16-18 mg/mL nicotine 70 mL	2.0–56.1 µg/150 puffs [0.013–0.374 µg/puff; 0.186–5.34 mg/m^3]	1.1-13.6 µg/150 puffs [0.007–0.091 µg/puff; 0.1–1.3 mg/m^3]	0.7-41.9 µg/150 puffs [0.005–0.28 µg/puff; 0.071–4.0 mg/m^3]	
Hutzler et al. (2014)	First-generation E(N)NDS device with pre-filled cartridge 55 mL	200 µg/150 puffs [1.33 µg/puff; 24.2 mg/m^3]	300 µg/150 puffs [2.00 µg/puff; 36.4 mg/m^3]	100 µg/150 [0.67 µg/puff; 12.2 mg/m^3]	Propionaldehyde: 30 µg/150 puffs [0.2 µg/puff; 3.7 mg/m^3]

Kosmider et al. (2014)	eGo-3 clearomizer device (2.4 Ω heating element; 900 mAh battery, 3.4 V) 10 commercial liquids with carrier of either PG only, PG/glycerol, or glycerol only; 18–24 mg/mL nicotine; and flavourings 70 mL	49–59 ng/15 puffs [0.003–0.004 µg/puff; 0.043–0.057 mg/m^3]	20–107 ng/15 puffs [0.001–0.0071 µg/puff; 0.014–0.101 mg/m^3]		Acetone: 59–296 ng/15 puffs [0.004–0.02 µg/puff; 0.057–0.29 mg/m^3]; Butanal: 15–185 ng/15 puffs [0.001–0.012 µg/puff; 0.014–0.171 mg/m^3]; Benzaldehyde: 21–46 ng/15 puffs [0.0014– 0.0031 µg/puff; 0.02–0.044 mg/m^3]; Crotonaldehyde: 53 ng/15 puffs [0.0035 µg/puff; 0.05 mg/m^3]; Isovaleric aldehyde: 33–47 ng/15 puffs [0.0022–0.0031 µg/puff; 0.031–0.044 mg/m^3]; m-methybenzaldehyde: 39–94 ng/15 puffs [0.0026–0.0063 µg/puff; 0.037–0.09 mg/m^3]
	eGo-3 clearomizer device (2.4 Ω heating element; 900 mAh battery, 3.2-4.8 V)	(ng/15 puffs) 3.2 V 0.53 (PG), 0.02 (glycerol),	(ng/15 puffs) 3.2 V 0.41 (PG), 0.17 (glycerol),		(ng/15 puffs) Acetone 3.2 V

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
	Control liquids: 1.8% nicotine; either 100% PG, 100% glycerol, or 1:1 PG/glycerol; no flavourings 70 mL	0.13 (PG/glycerol) 4.8 V 17.6 (PG), 0.15 (glycerol), 27.0 (PG/glycerol)	0.43 (PG/glycerol) 3.2 V 4.23 (PG), 1.24 (glycerol), 1.73 (PG/glycerol)		1.68 (PG), 0.34 (glycerol), 0.73 (PG/glycerol) 4.8 V 3.94 (PG), 1.43 (glycerol), 7.59 (PG/glycerol)
Tayyarah and Long (2014)	3x blu eCigs (disposable), 2xSKYCIG (rechargeable), 18-24 mg/mL nicotine' 55 mL	< LOQ	0.19 µg/puff [3.5 mg/m^3] (1 product)		Total carbonyls <1 µg/puff [<18 mg/m^3]; Propionaldehyde: 0.11 µg/puff [2.0 mg/m^3] (1 product)

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
Geiss et al. (2015)	2 x second generation E(N)NDS (1 atomiser; 1 cartomizer); 2 E(N)NDS liquids, each with 9 mg/mL nicotine, various flavours: 1 PG/glycerol 1 glycerol only 35 mL	19.6–23.5 ng/puff [0.56–0.67 mg/m^3]	8.1–39.9 ng/puff [0.23–1.14 mg/m^3]		Acetone: 2.7–8.8 ng/puff [0.08–0.25 mg/m^3] Acrolein: 0.5–13.5 ng/puff [14–0.37 mg/m^3]; Propanal: 0.9–4.9 ng/puff [0.03–140 mg/m^3]; Glycerol-only E(N)NDS liquid produced higher levels of carbonyls (specifically acrolein and acetaldehyde) than PG/glycerol E(N)NDS liquid
Herrington and Myers (2015)	4x E(N)NDS (first generation) PG/glycerol E(N)NDS liquids with 12-18 mg/mL nicotine 40 mL			0.12–0.6 µg/puff [3–15 mg/m^3]	

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
Jensen et al. (2015)	Tank system, commercial E(N)NDS liquid with nicotine, variable voltage 50 mL	As formaldehyde-releasing agents: 3.3 V Not detected 5.0 V 380 ng/10 puffs [0.038 µg/puff; 0.6 mg/m^3]			
Laugesen (2015)	14 brands imported to New Zealand (2013) (9 cigalikes, 3 disposables, 2 cartomizers with tank and battery), 11–23 mg/mL nicotine (label) (except 1 product, 0 mg/mL nicotine) 70 mL	Mean: 1.07 µg/m^3 (range: 0.48–2.50 µg/m^3) in aerosol	Mean: 0.81 µg/m^3 (range: 0.58–1.52 µg/m^3) in aerosol	Mean: 1.06 µg/m^3 (range: 0.13–3.58 µg/m^3) in aerosol	
Flora et al. (2016)	4x MarkTen brand E(N)NDS produced in 2014, 1.5% nicotine; (55 mL, 4 s ?)	0.090-0.33 µg/puff [1.6-6.0 mg/m^3]	< LOQ (0.71 µg/puff [13 mg/m^3])	Not detected	Crotonaldehyde not detected

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
Margham et al. (2016)	Vype ePen (closed modular system with cartomizer, operated at 3.6 V); 'Blended Tobacco' E(N)NDS liquid (25% PG, 48% glycerol, 25% water, 1.86% nicotine, tobacco flavour); 55 mL	0.122 µg/puff [2.22 mg/m^3]	0.106 µg/puff [1.93 mg/m^3]	0.070 µg/puff [1.27 mg/m^3]	Glyoxal: 0.056 µg/puff [1.02 mg/m^3]; Methylglyoxal: 0.046 µg/puff [0.84 mg/m^3]
Sleiman et al. (2016)	eGO CE4 (single coil); Kangertech AEROTANK mini (dual coil) with Vision Spinner II batter, variable voltage, 3.3-4.8 V; liquid containing 50/50 PG/glycerol, 18 mg/mL nicotine, classic tobacco flavour 50 mL	53 (3.3 V), 45.7 (3.8 V), 35.0 (4.3 V), 97 (4.8 V) µg/puff [1060 (3.3 V), 914 (3.8 V), 700 (4.3 V), 1940 (4.8 V) mg/m^3]	10 (3.3 V), 9.2 (3.8 V), 31.8 (4.3 V), 50 (4.8 V) µg/puff [200 (3.3 V), 184 (3.8 V), 636 (4.3 V), 1000 (4.8 V) mg/m^3]	3 (3.3 V), 8.5 (3.8 V), 15.8 (4.3 V), 21.5 (4.8 V) µg/puff [60 (3.3 V), 170 (3.8 V), 316 (4.3 V), 420 (4.8 V) mg/m^3]	3.8 V Glycidol: 2.1 µg/puff [42 mg/m^3]; Acetol: 7.6 µg/puff [152 mg/m^3] Di-acetyl: 2.2 µg/puff [44 mg/m^3]

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
Talih et al. (2016)	NHALER 510 Atomizer with ego-T battery (3.4 V); PG-based liquid with flavour and 0 or 18 mg/mL nicotine; E(N)NDS use by "direct dripping" of liquid (dripping every 2, 3, or 4 puffs); 152 mL (19 mL/s for 8 s)	20–88 µg/15 puffs [1.3–5.9 µg/puff; 8.6–39 mg/m^3]	270–1172 µg/15 puffs [18–78.1 µg/puff; 118–514 mg/m^3]	< LOQ	Total aldehydes: 399–1873 µg/15 puffs [26.6–125 µg/puff; 175–822 mg/m^3]; Propionaldehyde: 52-315 µg/15 puffs [3.5–21 µg/puff; 23–138 mg/m^3]; Valeraldehyde: 29-92 µg/15 puffs [1.9–6.13 µg/puff; 12.5–40.3 mg/m^3]; Methacrolein, butyraldehyde: < LOQ; Crotonaldehyde: < LOD

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
Uchiyama et al. (2016)	10 brands of second generation E(N)NDS sold in Japan, with operating characteristics ranging from: 3.7–5.1 V, 1.6–2.8 Ω, 5.1–14 W; liquids with or without nicotine Highest levels of carbonyls were measured on use brand 'D', which was operated at 3.7 V, 2.5 Ω, 5.5 W. Mean emission levels for brand D are reported in the columns to the right. 55 mL	Brand 'D' 120 µg/10 puffs [12.0 µg/puff; 218 mg/m^3]	Brand 'D' 73 µg/10 puffs [7.3 µg/puff; 130 mg/m^3]	Brand 'D' 24 µg/10 puffs [2.4 µg/puff; 44 mg/m^3]	Brand 'D' The highest mean total carbonyl compound production was 350 µg/10 puffs [1.3–5.9 µg/puff; 24–107 mg/m^3]. As gas Propanal 20 µg/10 puffs [2.0 µg/puff; 33 mg/m^3]; Acetol 7.3 µg/10 puffs [0.73 µg/puff; 13 mg/m^3] As particulate Glyoxal 43 µg/10 puffs [4.3 µg/puff]; Methyl glyoxal 58 µg/10 puffs [5.8 µg/puff; 105 mg/m^3]

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
Farsalinos et al. (2017)	CE4 top coil atomizer, Innokin iTaste VV V3.0 variable voltage battery device and Halo Caf; e Mocha liquid with 6 mg/mL nicotine (intended to replicate the test materials used by Jensen et al., 2015); 60 mL	3.3 V 3.4 µg/10 puffs [0.34 µg/puff; 5.7 mg/m^3] 4.0 V 19.8 µg/10 puffs [1.98 µg/puff; 33 mg/m^3] 5.0 V 718 µg/10 puffs [71.8 µg/puff; 1197 mg/m^3]			
Ogunwale et al. (2017)	4 x first-generation E(N)NDS 'blu' products with cartridge (16 mg/mL nicotine) purchased online; 4.6 W; 91 mL	0.18–0.62 µg/10 puffs [0.018–0.062 µg/puff; 0.19–0.68 mg/m^3]	0.15-0.57 µg/10puffs [0.015-0.057 µg/puff; 0.16–0.63 mg/m^3]	0.02–0.24 µg/10 puffs [0.002–0.024 µg/puff; 0.022–0.26 mg/m^3]	Acetone: 1.29–6.21 µg/10 puffs [0.129–0.621 µg/puff; 1.42–6.82 mg/m^3]

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
	Second-generation EVOD2/KangerTech + iTasteVV V3.0 battery + 3 liquids purchased in-store (6 mg/mL nicotine) 91 mL	9.1 W 8.2–40.04 µg/10 puffs [0.82–4.00 µg/puff; 9.0–44 mg/m^3]; 16.6 W 820 µg/10 puffs [82.0 µg/puff; 901 mg/m^3]	9.1 W 13.3-63.1 µg/10puffs [1.33–6.31 µg/puff; 14.6–69.3 mg/m^3]; 16.6 W 532 µg/10 puffs [53.2 µg/puff; 585 mg/m^3]	9.1 W 1.6–5.8 µg/10 puffs [0.16–0.58 µg/puff; 1.8–6.4 mg/m^3]; 16.6 W 16 µg/10 puffs [1.6 µg/puff]	9.1 W Acetone: 1.3–12.5 µg/10 puffs [0.13–1.25 µg/puff; 1.4–13.8 mg/m^3]; 16.6 W Acetone: 809 µg/10 puffs [80.9 µg/puff; 889 mg/m^3]; Propionaldehyde: 18 µg/10 puffs [1.8 µg/puff; 20 mg/m^3]; Butyraldehyde: 14 µg/10 puffs [1.4 µg/puff; 15 mg/m^3]
Salamanca et al. (2017)	KangerTech Pro Tank II ‘glassomizer’, 2.2 Ω, used on 10 W and 15 W power settings; base liquid, 1/1 PG/glycerol 50 mL	10 W 1.20 µg/mg base liquid; 15 W 4.43 µg/mg base liquid			

Study	E(N)NDS product(s); Usage parameters (if stated); Puff volume	Level collected from and/or measured in aerosol [conversion for this paper]			
		Formaldehyde	Acetaldehyde	Acrolein	Others
Farsalinos et al. (2018)	Apollo Classic Tobacco liquid (50:50 PG/glycerol ; 18 mg/mL nicotine) or an equivalent liquid without flavouring (the latter for Nautilus Mini only); CE4v2 atomizers with eGo-type variable battery, at 3.8 V and 4.8 V; Nautilus Mini atomizer with EVIC VTC Mini variable-wattage battery device, at 9.0 W and 13.5 W; CE4v2 generated dry puffs at 3.8 V and 4.8 V; Nautilus Mini did not generate dry puffs 50 mL	In E(N)NDS liquid CE4v2 797 µg/g (3.8 V) 4260 µg/g (4.8 V) Nautilus mini (+flavour) 16.7 µg/g (9.0 W) 16.5 µg/g (13.5 W) Nautilus mini (no flavour) 13.5 µg/g (9.0 W) 9.9 µg/g (13.5 W)	In E(N)NDS liquid CE4v2 321 µg/g (3.8 V), 2156 µg/g (4.8 V) Nautilus mini (+flavour) 9.6 µg/g (9.0 W) 10.3 µg/g (13.5 W) Nautilus mini (no flavour) 3.2 µg/g (9.0 W) 1.8 µg/g (13.5 W)	In E(N)NDS liquid CE4v2 321 µg/g (3.8 V) 2156 µg/g (4.8 V) Nautilus mini (+flavour) 8.6 µg/g (9.0 W) 11.7 µg/g (13.5 W) Nautilus mini (no flavour) 4.1 µg/g (9.0 W) 1.8 µg/g (13.5 W)	

Data from studies listed in TOX/2018/16. Values reported in square brackets have been calculated using the published data.

26. Emissions of formaldehyde, acetaldehyde, and/or acrolein were measured in several studies, with a wide range of levels reported. Overall, levels tended to be lower in earlier studies, with some more-recent studies reporting higher levels, generally where E(N)NDS devices were used on high power settings. The highest levels were noted in the study of Sleiman et al. (2016), in which a single-coil eGO CE4 device/Vision Spinner II battery with CT e-liquid ('Classic tobacco': 50/50 PG/glycerol, 18 mg/mL nicotine), was operated under the following conditions: 4.8 V/2.6 Ω, 5 s puff, 50 mL puff volume, 600 mL/min flow-rate; collection in 50-puff blocks taken over 25 min.

27. Highest reported mean concentrations

Formaldehyde:	97 µg/50 cm^3 puff [equivalent to 1940 mg/m^3 in aerosol] (Sleiman et al. 2016)
Acetaldehyde:	50 µg/50 cm^3 puff [equivalent to 1000 mg/m^3 in aerosol] (Sleiman et al. 2016)
Acrolein:	21.5 µg/50 cm^3 puff [equivalent to 420 mg/m^3 in aerosol] (Sleiman et al. 2016).

Metals

Studies that measured levels of individual metals present in E(N)NDS aerosols were summarised in the COT discussion paper, TOX/2018/15. This paper noted that where metals are present in E(N)NDS aerosols, these elements appear to be derived from the E(N)NDS device structure itself rather than from the E(N)NDS liquid, although metals may leach into E(N)NDS liquid during storage. As different E(N)NDS devices do not all use the same materials, the presence and quantity of the different metals in E(N)NDS aerosol is likely to be related to the materials used in the construction of the particular device, and perhaps also to other factors, for example, build quality and age of the device. In general, studies have reported wide variations in the ranges of measurements noted, both between and within brands, and even between experimental repeats using the same product/device. Given this variation, and the fact that design and manufacture of E(N)NDS products is a rapidly developing area, data regarding levels of individual metals measured in E(N)NDS aerosols are not tabulated here. However, a summary of the levels identified can be found in TOX/2018/15.

Biomarkers of exposure to E(N)NDS

28. A brief literature search was performed to identify data on levels of biomarkers of exposure to tobacco-related toxicants associated with E(N)NDS use, in comparison with levels in users of other tobacco products and in nonusers of tobacco products, as described in Annex A. Twenty-four publications of relevance to biomarkers of exposure to E(N)NDS were noted. These publications described 7 randomised clinical studies, 3 non-randomised 'switching' studies, 10 cross-

sectional epidemiological studies, 2 studies that followed CC smokers attempting to switch to E(N)NDS, and 1 review article, and 1 workshop proceedings.

29. Overall, studies tended to report that exposure to tobacco-related toxicants is lower from E(N)NDS use than from CC smoking, although levels of some toxicants (or their metabolites) may still be higher than levels measured in nonusers of tobacco products. While some studies reported lower levels of nicotine or nicotine metabolites associated with E(N)NDS use compared with CC smoking, others indicated equivalent levels of nicotine exposure from either product type. One study reported a higher nicotine level associated with use of E(N)NDS or of nicotine replacement therapy (NRT) than with CC smoking. This literature is summarised in the following sections.

Randomised clinical studies

30. Some short-term clinical studies have been carried out to assess the use of different tobacco products (including CC, E(N)NDS, nicotine gum or other noncombustible tobacco products, alone or used in combination) affects biomarkers of tobacco-related toxicants. These studies have generally reported substantially lower levels of biomarkers associated with E(N)NDS use compared with CC smoking. In some cases, levels of some toxicants associated with E(N)NDS were higher than levels measured in nonusers of tobacco products.

31. Landmesser et al. (2019)[19] conducted a randomised, controlled clinical study to assess the utility of isotope-labelled E(N)NDS constituents (PG, glycerol, nicotine) in biomonitoring studies. The study recruited 5 regular current CC smokers and 20 regular E(N)NDS users, who did not use other tobacco or nicotine-containing products. In the first part of the study (clinically confined), participants in the E(N)NDS group were randomised to use a tank-style E(N)NDS product (50:50 PG:glycerol, 12 mg/mL nicotine, tobacco flavour) spiked with 10% labelled PG, glycerol, and nicotine at either 18 W or 10 W power for 10 pre-defined 10-puff vaping sessions over a period of 1 day. Consumption level of E(N)NDS liquid was not significantly different between the 2 groups (1.26 and 1.56 g/10 sessions in 10 W and 18 W groups, respectively). CC smokers smoked 10 non-filter CC (0.32 mg nicotine per CC) (1 CC per session) spiked with labelled PG, glycerol, and nicotine. Unlabelled and labelled forms of PG, glycerol, nicotine and nicotine metabolites were determined in plasma, urine, and saliva at various time points during the test exposures and the following 3 days.

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[19] Declarations. *Funding*: “This research was supported by Altria Client Services”. *Declaration of Interests*: “The authors GS, MS, NP, and AL work for ABF GmbH, Munich, an independent contract research laboratory and declare no conflicts of interest regarding the publication of this paper. The authors M. Sarkar and JE are employed by Altria Client Services and declare no conflicts of interest regarding the publication of this paper”.

32. Nicotine was virtually undetectable in plasma at time zero, and peaked with each test session. Nicotine was detected at ratio of approximately 10/1 unlabelled/labelled during the test sessions with E(N)NDS, was higher in CC smokers than E(N)NDS users, and showed higher levels in 18 W than 10 W E(N)NDS users. Total (labelled + unlabelled) peak plasma nicotine levels were 15.8 ng/mL (E(N)NDS, 10W), 19.6 ng/mL (E(N)NDS, 18 W), and 36.0 ng/mL (CC). These were considered to be similar to the ranges reported in the literature. No nicotine was detected in plasma 12 h after the final test session (Landmesser et al. 2019).

33. Labelled and unlabelled cotinine were detected in plasma and urine and maximum plasma cotinine was achieved around 2 h after the final test exposure for all groups (calculated as total concentrations of 31, 39, and 2.5 ng/mL in 10 W E(N)NDS, 18 W E(N)NDS, and CC, respectively) . A similar pattern was seen in saliva (Landmesser et al. 2019).

34. In urine, excretion of labelled total nicotine equivalents (nicotine plus cotinine and other major metabolites) was still present 72 h after session 10 (Landmesser et al. 2019).

35. Labelled and unlabelled PG were detected in plasma at peaks after test sessions. The levels were higher in E(N)NDS groups compared with the CC smoker group. Maximum plasma PG concentrations after E(N)NDS use were observed after the 10th test session. The ratio of unlabelled to labelled PG was higher than 10, assumed to be due to background PG from other sources, and a background level of around 1 µg/mL unlabelled PG was observed. Within 13 h of the final test session, labelled PG was undetectable in plasma. No relation to the E(N)NDS test session was observed for PG in saliva. In urine, peak excretion of PG (labelled and unlabelled) occurred shortly after the 10th test session (Landmesser et al. 2019).

36. For glycerol, plasma levels of labelled (< LOD) and unlabelled (30–40 µg/mL) forms did not change in relation to test sessions. No relation to E(N)NDS test session was observed for glycerol in saliva. Labelled glycerol was not detected in urine of any of the test groups and unlabelled glycerol in urine did not show a relation to test sessions (Landmesser et al. 2019).

37. Authors concluded that the inclusion of 10% of labelled nicotine and PG in E(N)NDS liquid is suitable for quantifying biomarkers of exposure to these ingredients. However, the inclusion of labelled glycerol is not useful, probably due to the high physiological levels of glycerol as well as the rapid metabolism. Saliva was not considered to be a suitable medium for measurement of PG and glycerol as internal dose biomarkers, due to external presence of these compounds in the oral cavity as a result of vaping (Landmesser et al. 2019).

38. Lorkiewicz et al. (2018) measured levels of several urinary biomarkers in self-reported occasional users of various tobacco products: CC (n = 4 sole users); smokeless tobacco (ST) (chewing tobacco, dry or moist snuff) (n = 10); first-

generation E(N)NDS or mentholated E(N)NDS (n = 3 sole users). Subjects in the control group (n = 12 non-users) did not use tobacco products or have exposure to secondhand smoke. Two baseline urine samples were taken, 24 h apart. After this, participants were instructed to abstain from any tobacco product use for 48 h, following which a urine sample was collected (time 0). Participants then either smoked 1 CC (1.2 mg/CC nicotine), used 1 or 2 pouches of ST (10.5 mg/g nicotine) ad libitum, or used an E(N)NDS product (2.4% nicotine) or an E(N)NDS product with menthol (3.0% nicotine) ad libitum for 15 min (minimum 15 puffs). Urine samples were collected at times 20, 40, 80, 120 and 180 min after time 0, and analysed for total alkaloids (TA – nicotine, anatabine, anabasine), cotinine, 3-hydroxycotinine (3-HC), and 20 VOCs.

39. At baseline, urinary nicotine was significantly higher in the CC and ST groups than the control group (in whom the level was zero), while only trace levels of nicotine were measured in the E(N)NDS group. Urinary cotinine and 3-HC were detected in all groups except controls. Compared with controls, metabolites of xylene, N,N-dimethylformamide, acrylonitrile, and crotonaldehyde were significantly higher in urine of CC users, while baseline urine of E(N)NDS users showed higher levels of xylene, cyanide, styrene, ethylbenzene, acrolein, and benzene metabolites. Levels of VOC metabolites in the urine of ST users were comparable with controls (Lorkiewicz et al. 2018).

40. At time 0, nicotine levels were very low in all groups. Cotinine and 3-HC were still present. Anatabine was detected in urine of CC and ST groups, but not E(N)NDS users (Lorkiewicz et al. 2018).

41. During test product use, urinary nicotine elimination occurred rapidly in all groups (max 20 min in CC group, 40 min in ST and E(N)NDS groups), and cumulative nicotine collection was approximately 2-fold in CC compared with ST or E(N)NDS groups. Cumulative cotinine and 3-HC were the same in all groups. Cumulative anatabine was similar in CC and ST groups, but not detected in E(N)NDS (Lorkiewicz et al. 2018).

42. Compared with controls, authors reported that E(N)NDS users had higher levels of urinary metabolites of xylene, cyanide, styrene, ethylbenzene, and benzene at baseline and elevated urinary levels of metabolites of xylene, N,N-dimethylformamide, and acrylonitrile after E(N)NDS use. Metabolites of acrolein, crotonaldehyde, and 1,3-butadiene were significantly higher in CC smokers than users of other products or controls. VOC metabolite levels in ST users were similar to those in nonusers, except for the xylene metabolite, 2-methylhippuric acid (2-MHA), which was around 3-fold higher than in controls (Lorkiewicz et al. 2018).

43. Round et al. (2018)[20] compared levels of biomarkers of exposure to nicotine and tobacco-related toxicants in 153 CC smokers randomised to switch to 1 of 4 groups of substitute product use for 6 days. Smokers of non-menthol CC were randomised to switch to either 'VS brand' E(N)NDS product (PG, glycerol, 4.8% nicotine, water, original flavour), or to nicotine gum (NG) (4 mg/gum). Smokers of menthol CC were randomised to switch to either VS brand E(N)NDS product (PG, glycerol, 4.8% nicotine, water, menthol flavour), or to NG (4 mg/gum). Participants were confined to the clinic and products were dispensed by staff. Products were used ad libitum on days -3 and -2 (own-brand CC) and on days 1–5 (test product). On each of these days, ad libitum use was followed by a 12-h abstinence (overnight). Nicotine pharmacokinetic evaluations were performed over a 6 h period on the morning of day -1 and of day 6. During these evaluations, participants smoked own-brand CC (day -1) or used the test product (day 6). Blood samples were collected on days -2, 1, 3, 5. Urine samples (24 h) were collected from days -3 to -2, and days 4 to 5. Biomarkers for the following chemicals were evaluated: total nicotine equivalents, CO, benzene, acrolein, crotonaldehyde, 1,3-butadiene, acrylonitrile, ethylene oxide, NNK, NNN, NAT, NAB, 1-aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl, 4-aminobiphenyl, o-toluene, naphthalene, benzo[a]pyrene, fluorine, pyrene, acrylamide, hydrogen cyanide, general mutagenic properties of urine.

44. Compared with baseline, mean total urinary nicotine equivalents at the end of the test period had decreased significantly, by 38%, in both E(N)NDS groups, and by 60% and 67%, respectively, in the non-menthol-smoker and menthol-smoker NG groups. Levels of biomarkers of all toxicants showed a statistically significant[21] decrease in all groups at the end of the test period compared with baseline. The authors noted that "Biomarkers of toxicants decreased 30%–99% for all groups, and generally decreased by similar amounts whether subjects were switched to an e-cigarette or NG". However, between-group statistical analyses were not reported. Authors concluded that nicotine exposure was maintained closer to levels achieved by CC smoking by using the E(N)NDS product tested in this study than nicotine gum, and that biomarkers of tobacco consumption decreased to similar levels in both E(N)NDS and nicotine gum groups (Round et al. 2018).

[20] Declarations. *Funding*: "This study was funded by R.J. Reynolds Vapor Company through R.J. Reynolds Tobacco Company". *Declaration of Interests*: "ER, PC, and ES are full-time employees of RAI Services Company. AT was a full-time employee of RAI Services Company at the time this study was conducted. RAI Services Company is a wholly owned subsidiary of Reynolds American Inc., which is a wholly owned subsidiary of British American Tobacco plc".

[21] Except for 3-hydroxy-benzo[*a*]pyrene in the non-menthol-smoker nicotine gum group, for which the decrease was not statistically significant.

45. D'Ruiz, Graff and Robinson (2016)[22] reported that complete substitution of CC with E(N)NDS over a short time period led to a reduction in exposure to harmful tobacco-related toxicants and carcinogens (with the exception of nicotine) that was similar in magnitude to that achieved by quitting CC smoking (without E(N)NDS use). Switching to dual CC/E(N)NDS use led to a lesser level of reduction. In this open-label, forced-switch, parallel-arm study, 103 regular CC smokers were randomised to 1 of 7 groups (n = 16 per group): Groups A1, A2, and A3 (E(N)NDS with different flavour and/or device type); Groups B1, B2, and B3 (dual use: E(N)NDS product as for groups A1, A2, or A3, respectively, + 50%-level use of own-brand CC); Group C (complete nicotine cessation). E(N)NDS products contained varying percentages of PG and glycerol, 24 mg/mL nicotine, distilled water, and flavours. The study was conducted for 5 days under clinical confinement, with use of the specified product allowed ad libitum from 07:00 to 23:00 each day. Exhaled breath carbon monoxide (CO) and nitric oxide (NO) were measured daily, each afternoon, over a period of 1 week. Urine was collected as 24-h samples and blood was sampled each evening. The following biomarkers were measured (biomarker / chemical constituent). In urine: (4-(methylnitrosamino)-1–(3-pyridyl)-1-butanol) (NNAL) / NNK; 3-hydroxy-propylmercapturic acid (3-HPMA) / (acrolein); 3-hydroxy-1-ethylpropylmercapturic acid (HMPMA) / crotonaldehyde; 2-cyanoethylmercapturic acid (CEMA) / (acetonitrile); 1-hydroxypyrene (1-OHP) / pyrene; NNN (NNN); Monohydroxy-3-butenyl mercapturic acid (MHBMA) / 1,3-butadiene; S-phenylmercapturic acid (S-PMA) / benzene; nicotine equivalents (nicotine). In blood: COHb (CO); plasma nicotine (nicotine); plasma cotinine (nicotine), plasma trans-3'hydroxycotinine (nicotine).

46. In the 'B' groups, CC consumption reduced by approximately one-third compared with monitoring the day before the study (Day -1). Although all 'A' groups used more E(N)NDS than 'B' groups, the increase was only statistically significant for 1 of the 2 flavours tested (cherry, but not tobacco). Statistically significant, positive linear relationships were observed between percent change (reduction) in biomarker excretion and the percent change in CC per day smoked, for all urine biomarkers except nicotine equivalents. The level of changes between the cessation and E(N)NDS-only groups were not significantly different, except for urinary and blood markers of nicotine. All biomarker level changes in dual-use groups were significantly less compared with those in the cessation group. All groups showed

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[22] Declarations. *Funding*: *"*This study was funded by Fontem Ventures B.V., a fully owned subsidiary of
Imperial Brands plc, and the manufacturer of the e-cigarette products used in this study." *Competing interests*: "CDD is currently an independent clinical study consultant to Fontem, U.S. and former full-time employee of Lorillard and ITG Brands, LLC; former and current affiliates of the study sponsor. ER is a full-time employee of ITG Brands, LLC, an affiliate of the study sponsor. DWG is an employee of Celerion, the contract research organization (CRO) that conducted the study."

statistically significant decreases in exhaled CO at Day 5 compared to Day -1, with decreases in the cessation and E(N)NDS-only use groups ranging from approximately 88–89% (no significant difference between cessation and E(N)NDS-only) and in the dual use groups from approximately 26–32% (significant difference compared with cessation). Exhaled NO increased in cessation and E(N)NDS-only groups by approximately 46–63%, but not in dual users. Blood COHb was significantly reduced at Day 5 compared with Day -1 in the cessation and E(N)NDS-only groups, with no significant difference between these 2 groups (D'Ruiz et al. 2016). This study and these data were also reported in another publication by some of the same authors (O'Connell, Graff and D'Ruiz 2016)[23].

47. A non-blinded, within-subject, crossover study compared levels of exhaled CO and urinary biomarkers associated with use of CC and/or E(N)NDS (exclusive, dual, or neither) in 48 regular daily dual CC/E(N)NDS users (Czoli et al. 2018)[24]. For E(N)NDS use, 92% of participants used tank systems and 94% used products containing nicotine (of which, 71% ≤ 14 mg/mL). Participants were randomised to protocols of back-to-back 7-day periods of product use as follows. Group A: dual use, E(N)NDS use, CC use, no product use. Group B: dual use, CC use, E(N)NDS use, no product use. Urine samples were collected at baseline and after each 7-day test period. General product use habits were recorded by questionnaire, and daily product use diaries were completed by participants during the study. Overall, 54–58% of participants smoked CC during periods when this was prohibited by the study protocol and 25–31% used E(N)NDS during periods when this was not permitted. The following biomarkers were measured: CO in exhaled breath; urinary cotinine, 1-HOP (metabolite of pyrene, a PAH), and NNAL (metabolite of NNK) (adjusted for creatinine).

48. For cotinine, compared with dual use (the baseline condition), levels were not significantly different during CC-only use, but decreased significantly during exclusive E(N)NDS (-38%) and no product (-26%) use. There were no significant differences between cotinine levels during E(N)NDS-only or no product use. Exhaled breath CO was significantly lower during E(N)NDS-only (-41%) or no product (-26%) use than dual use, while levels increased during CC-only use (+21%). There were no

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[23] Declarations. *Funding*: "The work in this manuscript was supported by Fontem Ventures B.V., a fully owned subsidiary of Imperial Brands plc, and the manufacturer of the e-cigarette products used in this study". *Disclosure statement*: "CDD is consultant for Fontem Ventures U.S. Inc.; GOC is an employee of Fontem Ventures, B.V.; DWG is an employee of Celerion, the contract research organization (CRO) that conducted the study".

[24] Declarations. *Declaration of Interests*: "MLG reports grants from and served as an advisory board member to pharmaceutical companies that manufacture smoking cessation drugs. DH has provided paid expert testimony
in tobacco litigation on behalf of governments and class-action plaintiffs on issues related to tobacco product science and regulation. The other authors have no competing interests to declare."

significant differences between CO levels during E(N)NDS-only or no product use. Compared with dual-use, levels of 1-HOP were increased (+23%) during CC-only use and decreased during E(N)NDS-only (-31%) or no product (-14%) use. Levels were significantly higher during E(N)NDS-only than no product use. Compared with dual-use, levels of NNAL were not significantly increased during CC-only use, and significantly decreased during E(N)NDS-only (-30%) or no product (-35%) use. There were no significant differences between levels during E(N)NDS-only or no product use. Authors concluded that E(N)NDS are not harmless, but are less harmful than CC, and that use of E(N)NDS while smoking CC may not necessarily reduce health risks, thus consumers should completely stop smoking CC to maximise health benefits (Czoli et al. 2018).

49. An open-label, randomised, parallel-group, clinical study was performed to evaluate various aspects related to switching from CC smoking to E(N)NDS use. This included clinical and psychological parameters, adverse events, biomarkers of exposure and of biological effect (Cravo et al. 2016)[25]. A total of 387 participants, all regular CC smokers, completed the study. Of these, 101 were randomised to continue smoking own-brand CC and 286 were randomised to switch to using an E(N)NDS product (containing 70-75% PG, 18-20% glycerol, 5% water, 2.0% nicotine, and flavour) for a period of 12 weeks. Urine samples were collected at baseline and at weeks 4, 8, and 12. The following biomarkers were measured: nicotine equivalents (nicotine, cotinine, nicotine-N-glucuronide, cotinine-N-glucuronide, trans 3'-hydroxycotinine and trans 3'-hydroxycotinine glucuronide); S-PMA (biomarker of exposure to benzene); 3-HPMA (biomarker of exposure to acrolein); PG; total NNAL (NNAL + NNAL-glucuronide) (biomarker of exposure to NNK). Subjects in the E(N)NDS arm used a mean 3.29–4.25 capsules per day, and mean self-reported CC consumption in this group was 1.43–1.86 CC/day. Participants in the CC arm used a mean 12.33–14.1 CC/day. In the E(N)NDS group, levels of all urinary biomarkers except PG were lower than baseline at all measured study time points, with levels generally lowest at 4 weeks and then increasing slightly thereafter (at 4 weeks, nicotine equivalents, -33.8%; 3-HPMA, -34.5%; S-PMA, -54.5%; total NNAL, -43.5%). Urinary PG in this group increased over baseline, in proportion to the number of E(N)NDS capsules used (+182.5% at week 4, +166.7% at week 8, +119.2% at week 12). Levels of all biomarkers, including PG, did not change significantly over time in the CC group. A sub-study conducted on a small group of the participants in a situation of monitored clinical confinement during week 1 of the 12-week study also showed a similar pattern in terms of levels of urinary biomarkers in relation to exposure group, although the overall levels of biomarkers in these subjects were lower at baseline.

[25] Declarations. *Funding*: "This work was funded and supported by Fontem Ventures B.V. Imperial Brands plc is the parent company of Fontem Ventures B.V., the manufacturer of the EVP prototype used in this study."

Non-randomised switching studies

50. Three studies noted decreased levels of most tobacco-related toxicants in subjects who were followed during a period during which they attempted to switch from CC smoking to E(N)NDS use.

51. McRobbie et al. (2015)[26] evaluated levels of exhaled CO and urinary biomarkers of nicotine (cotinine) and acrolein (3-HPMA) in 34 CC smokers during a 4-week period during which the aim was to abstain from CC smoking and instead use E(N)NDS (greenesmoke cigalike containing PG, glycerol, and 2.4% nicotine). Measurements were taken at baseline 1 week prior to target quit date (TQD) and at 4 weeks post TQD. At the end of the 4-week study period, 1 participant was using CC only, 17 participants were dual-using CC and E(N)NDS, and 16 participants were using E(N)NDS only. There was no significant difference in average levels of E(N)NDS use between the E(N)NDS or CC/E(N)NDS groups during week 4. At baseline, levels of CO, cotinine, and 3-HPMA were significantly higher in the group of 17 participants who finished the study as dual users than in the group of 16 who used only E(N)NDS at the end of the study. Compared with baseline, significant decreases were noted in (dual, E(N)NDS-only) exhaled breath CO (-52%, -80%) and 3-HPMA (-60%, -79%). Mean baseline urinary cotinine also decreased significantly from baseline in dual users (-44%, $p = 0.010$), while a non-significant decrease was noted in E(N)NDS-only users (-17%, $p = 0.486$).

52. Goniewicz et al. (2017)[27] measured urinary biomarkers for nicotine and tobacco-related toxicants (TSNA – NNK; VOCs – 1,3-butadiene, crotonaldehyde, acrolein, benzene, acrylamide, acrylonitrile, ethylene oxide, propylene oxide; PAHs – naphthalene, fluorene, phenanthrene, and pyrene) in 20 CC smokers, in samples collected before and after switching to an E(N)NDS product (50% PG, 50% glycerol, 11.0 ± 1.5 mg/cartridge nicotine, tobacco flavour) for 2 weeks. Participants were provided with 20 cartridges per week. In total, 45% of participants reported complete

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[26] Declarations. *Disclosure of Potential Conflicts of Interest*: “H. McRobbie is Clinical Director at The Dragon Institute; reports receiving commercial research grant from Pfizer; and has received speakers bureau honoraria from Johnson & Johnson and Pfizer. M.L. Goniewicz reports receiving commercial research grant from Pfizer. P. Hajek has received speakers bureau honoraria from and is a consultant/advisory board member for the manufacturers of stop-smoking medications. No potential conflicts of interest were disclosed by the other authors”.

[27] Declarations. *Declaration of Interests*: “MLG was a faculty member of the Medical University of Silesia, Poland during the study. He received a research grant from Pfizer, a pharmaceutical company that markets smoking cessation medications. MLG and NLB have been consultants to pharmaceutical companies that market smoking cessation medications. NLB has been an expert witness in litigation against tobacco companies. The other authors declare no potential conflicts of interest”.

abstinence from cigarette smoking at 2 weeks, while 55% reported continued smoking.

53. Exhaled CO was significantly decreased at weeks 1 and 2 compared with baseline. Levels of total nicotine and metabolites for the PAHs, phenanthrene, pyrene, and naphthalene, did not change significantly after switching from CC to E(N)NDS. All other biomarkers significantly decreased after 1 week of using E(N)NDS, with the greatest percentage reductions in biomarker levels observed for metabolites of 1,3-butadiene, benzene, and acrylonitrile. Total NNAL (NNK metabolite) had decreased by 57% and 64% after 1 and 2 weeks, respectively, compared with baseline, while 3-hydroxyfluorene levels had decreased by 46% at week 1, and 34% at week 2 (Goniewicz et al. 2017).

54. In another study, urinary cotinine, NNAL, and metabolites of 8 VOCs (benzene, ethylene oxide, N-nitrosodimethylamine, acrylonitrile, acrolein, propylene oxide, acrylamide, crotonaldehyde) were measured at baseline and after 4 weeks in 40 CC smokers provided with E(N)NDS products (glycerol, 12 mg/mL or 24 mg/mL nicotine, flavour) as an optional alternative to CC smoking (Pulvers et al. 2018)[28]. All 37 participants with follow-up data reported using the study E(N)NDS product. Overall average reduction in CC consumption was 7.1 CC per day at week 1, with 16 and 6 participants, respectively, reporting no CC smoking at weeks 2 and 4. There was no significant change in nicotine intake over the 4-week period. In the whole study group, exhaled CO, NNAL, and metabolites of benzene and acrylonitrile were significantly decreased at week 4 compared with baseline. CC smokers who reported switching exclusively to E(N)NDS for at least half of the study period demonstrated significant reductions in metabolites of ethylene oxide and acrylamide.

Cross-sectional studies

55. Several evaluations have been published recently that have investigated correlations between levels of biomarkers of exposure to tobacco-related toxicants in biological samples collected from participants in population–based studies and data collected on their use of different types of tobacco products. In general, in concurrence with clinical studies, these investigations have indicated levels of tobacco-related toxicants substantially lower in E(N)NDS users than CC smokers, although not always as low as levels in nonusers of tobacco products. Most, but not all, studies indicated lower levels of toxicants in dual CC/E(N)NDS users compared with CC smokers, or reductions in toxicant levels in CC smokers implementing E(N)NDS use towards the aim of reducing or quitting CC smoking.

[28] Declarations. *Declaration of Interests*: “Benowitz is a consultant to pharmaceutical companies that market smoking cessation medications and has been an expert witness in litigation against tobacco companies. The other authors have no conflicts of interest”.

56. Using data from the 2013–2014 US 'Population Assessment of Tobacco Health' (PATH) study, Goniewicz et al. (2018)[29] compared levels of tobacco-related toxicants in participants who had provided a spot urine sample and who were either exclusive E(N)NDS users (n = 247), exclusive CC users (n = 2411), dual users of E(N)NDS and CC (n = 792), or never users of tobacco products (n = 1655). Levels of daily CC use were similar in the exclusive-CC and dual-CC/E(N)NDS groups. The percentage of subjects using E(N)NDS daily was greater for exclusive E(N)NDS users than for dual E(N)NDS/CC users. A total of 50 biomarkers covering 5 classes of tobacco-related chemicals were evaluated, including nicotine, TSNAs, metals, PAHs, and VOCs. Compared with E(N)NDS-only users, never-users of tobacco products had significantly lower concentrations of biomarkers of exposure to nicotine, NNK (-81%), some metals (lead, -19%; cadmium, -23%), and some VOCs (pyrene, - 20%; acrylonitrile, -66%). Compared with CC-only users, E(N)NDS-only users showed significantly lower concentrations of biomarkers of exposure including total nicotine equivalents (-93%), NNAL (-98%), cadmium (-30%) PAHs (naphthalene, - 62%; pyrene, - 47%), and most VOCs (acrolein, -60%; acrylonitrile, -97%; acrylamide, - 59%). CC-only users showed significantly lower concentrations of some biomarkers than dual users (total nicotine equivalents, -36%; NNAL, -23%; pyrene, -15%; acrolein, -10%; acrylonitrile, -15%), while other biomarkers did not differ significantly between these 2 groups. Frequency of CC use among dual users was positively correlated with nicotine and toxicant exposure. Authors concluded that exclusive use of E(N)NDS is associated with measurable exposure to known tobacco-related toxicants, but generally at lower levels than CC smoking, with toxicant exposure highest in dual users.

57. Wei et al. (2018)[30] used data from a subset of 1572 participants in the 2013–2014 US National Health and Nutrition Examination Surveys (NHANES) survey to evaluate correlations of urinary metabolites of phosphate-based flame retardants (PFRs) with use of E(N)NDS and other tobacco products. Diphenyl phosphate (DPhP), bis(1,3-dichloro-2-propyl) phosphate (BDCPP), bis(2-chloroethyl) phosphate (BCEP), and dibutylphosphate (DBUP) were detected in all E(N)NDS users. The adjusted geometric mean level of BCEP, the metabolite of tris(2-chloroethyl) phosphate (TCEP), was 81% higher in E(N)NDS users compared with nonusers of any tobacco product ($p = 0.0124$) and significantly higher compared with levels in CC users or cigar users ($p < 0.05$). Authors commented that the findings of the study suggested that certain PFRs may be present in E(N)NDS as contaminants, leading

[29] Declarations. *Conflict of Interest Disclosures*: "Dr Goniewicz receives fees for serving on an advisory board from Johnson & Johnson and grant support from Pfizer. No other disclosures were reported".
[30] Declarations. *Conflicts of Interest*: "M.L.G. received a research grant from Pfizer and served as a member of advisory board to Johnson & Johnson, manufacturers of smoking cessation medication. The other authors declare no actual or potential competing financial interests".

to higher levels of exposure in users than nonusers, but they noted that a limitation was the small number of E(N)NDS users in the survey (n = 14).

58. Using data from the US 'Population Assessment of Tobacco and Health' (PATH) study, Wang et al. (2019) reported that users of tobacco products (in which E(N)NDS were included) had higher PAH urinary biomarker concentrations compared with never users, with concentrations differing by type and frequency of tobacco product use. Seven PAH urinary biomarkers were quantified in a set of 8327 participants who had reported their habits of tobacco product use as either never user (n = 1700), current exclusive use of combustible product (n = 5767), or current exclusive use of non-combustible product (n = 860). Tobacco product users were categorised as 3964 exclusive CC users, 509 smokeless tobacco (SLT) users, and 280 E(N)NDS users. Combustible product users had significantly higher mean levels of all biomarkers compared with users of noncombustible products or with never users. SLT users had significantly higher mean levels of 4 of 7 biomarkers compared with never users. SLT users had significantly higher levels of 2-hydroxyfluorene, 3-hydroxyfluorene and ∑2,3-hydroxyphenanthrene compared with E(N)NDS users. E(N)NDS users had significantly higher levels of 3-hydroxyfluorene and 1-OHP compared with never users.

59. Rubinstein et al. (2018)[31] evaluated levels of salivary cotinine and urinary NNAL and 8 VOCs in adolescent (average age, 16.4 y) E(N)NDS-only users, dual E(N)NDS and CC users, and controls (never use of either product; urinary NNAL level consistent with no exposure to CC smoking or exposure to secondhand smoke) (n = 20). E(N)NDS-only users used the product a mean of 12.8 days/month, and salivary cotinine levels were consistent with levels of use reported for the previous 30 days. Dual users used E(N)NDS a mean of 25.5 days/month. For E(N)NDS users, screening appointments at which samples were collected were all conducted within 24 h of E(N)NDS use. Median urinary NNAL levels were 0, 0.3, and 68.1 pg/mL creatinine in controls, E(N)NDS-only users, and dual users, respectively. Median levels of other biomarkers were reported as follows (controls, E(N)NDS-only users, dual users; values in ng/mg creatinine): PMA (benzene) (0, 0, 0.2); MHBMA (1,-3-butadiene) (0, 0, 0); HEMA (ethylene oxide) (1.3, 0.5, 1.0); CNEMA (acrylonitrile) (0, 1.3, 59.4); 3-HPMA (propylene oxide) (15.2, 28.8, 40.2); AAMA

[31] Declarations. *Financial Disclosure*: "Dr Benowitz is a consultant to several pharmaceutical companies that market medications to aid smoking cessation and has served as a paid expert witness in litigation against tobacco companies. Drs Ramo and Rubinstein have consulted for Carrot Inc, which makes a tobacco cessation device; and Dr Delucchi has indicated he has no financial relationships relevant to this article to disclose". *Potential Conflict of Interest*: "Dr Benowitz is a consultant to several pharmaceutical companies that market medications to aid smoking cessation and has served as a paid expert witness in litigation against tobacco companies. Drs Ramo and Rubinstein have consulted for Carrot Inc, which makes a tobacco cessation device; and Dr Delucchi has indicated he has no potential conflicts of interest to disclose".

(acrylamide) (34.5, 67.3, 235.6); HMPMA (crotonaldehyde) (100.4, 148.7, 185.4). Biomarkers for benzene, ethylene oxide, acrylonitrile, acrolein, and acrylamide were significantly higher in dual CC/E(N)NDS users compared with E(N)NDS-only users. Biomarkers for acrylonitrile, acrolein, propylene oxide, acrylamide, and crotonaldehyde were significantly higher in E(N)NDS-only users compared with controls. Authors also noted that biomarkers for acrylonitrile and acrylamide were significantly higher in participants who reported using nicotine-containing E(N)NDS all or some of the time compared with those who use nicotine-free products or were not sure. Biomarker for acrylonitrile was also significantly higher in users of fruit-flavoured E(N)NDS products. Authors concluded that E(N)NDS aerosol may be less hazardous than CC smoke, with lower overall exposure to VOC toxicants, although exposure is not risk free and messaging to teenagers should include warnings of potential risk from toxic exposure to carcinogenic compounds generated by these products.

60. Hecht et al. (2015) found that urinary levels of 1-HOP, total NNAL, 3-HPMA, 2-HPMA, HMPMA, and S-PMA were significantly lower in 28 E(N)NDS users compared with levels reported in the literature for CC smokers, in 3 studies that had used the same assay methods. Levels of nicotine and cotinine were either similar to or lower than levels reported in the literature for CC smokers, depending on the study used for comparison. E(N)NDS users in the study of Hecht et al. (2015) were ex-CC smokers who had used E(N)NDS products (different brands, average nicotine concentration, 12.5 mg/mL), for a median of 9 months.

61. A study reported by Shahab et al. (2017)[32] assessed biomarkers of exposure to nicotine and tobacco-related toxicants associated with CC smoking, E(N)NDS use, and NRT. Participants in the study were either current CC smokers (smoked CC for ≥ the prior 6 months) or ex-CC smokers (not smoked CC for ≥ the prior 6 months), all with a long-term history of CC smoking. The following 5 user groups were specified (n = 36 or 37 per group): CC-only; dual CC and NRT; dual CC and E(N)NDS; NRT only; E(N)NDS-only. E(N)NDS or NRT use was specified as use of a product containing nicotine for ≥ the prior 6 months. Daily usage levels of E(N)NDS or NRT were higher in single-use than dual-use groups. CC smoking levels by group were reported as follows (CC per day): CC-only (13.9), dual CC and NRT (10.8),

[32] Declarations. *Conflict of Interest*: “LS has received an honorarium for a talk, an unrestricted research grant and travel expenses to attend meetings and workshops from Pfizer, a pharmaceutical company that makes smoking cessation products, and has acted as paid reviewer for grant awarding bodies and as a paid consultant for health care companies. MLG reports research grants from and served as an advisory board member to pharmaceutical companies that manufacture smoking cessation medications. JB has received unrestricted research funding from Pfizer to study smoking cessation. RW has received travel funds and hospitality from, and undertaken research and consultancy for, pharmaceutical companies that manufacture or research products aimed at helping smokers to stop. The other authors have no conflicts of interest to declare”.

dual CC and E(N)NDS (11.9), NRT-only (14.7), E(N)NDS only (15.9). (It is assumed that daily CC consumption reported for ex-smoker groups refers to levels prior to quitting CC smoking, although this was not actually stated in the report). Saliva and/or urine samples were analysed for biomarkers of nicotine, TSNA (NNK), and VOCs (acrolein, acrylamide, acrylonitrile, 1,3-butadiene, and ethylene oxide).

62. Salivary and urinary nicotine levels did not vary significantly between groups. An analysis adjusted for a number of variables[33] reported biomarker levels as a proportion of those measured in the CC-only group, and indicated that levels of total nicotine equivalents were similar (although slightly higher) in all groups compared with CC-only (as a percentage of CC-only group level: NRT-only, 121.6%; E(N)NDS-only, 126.9%; dual CC and NRT, 104.2%; dual CC and E(N)NDS, 156.8%). Levels of the NNK biomarker, NNAL, were reduced in all groups compared with CC-only (as a percentage of CC-only group level: NRT-only, 11.6%; E(N)NDS-only, 2.5%; dual CC and NRT users, 57.1%; dual CC and E(N)NDS users, 81.2%). For VOCs, proportions compared with CC-only users were similar (between 80–120% of CC-only level) in both the dual CC/NRT and dual CC/E(N)NDS groups, but levels were generally lower in the single-product-use NRT-only and E(N)NDS-only groups (range 2.9–54.2% compared with the CC-only group). Authors concluded that exclusive, long-term use of NRT or E(N)NDS by ex-(CC)-smokers is associated with substantially reduced levels of selected carcinogens and toxicants as compared with CC smoking, although this does not appear to be the case when there is continued, concurrent use of CC. They also noted that this study indicated that E(N)NDS use was not associated with higher levels of carcinogens or toxicants than NRT use, and that nicotine delivery was approximately equivalent from all products evaluated.

63. Wagener et al. (2017) compared levels of salivary cotinine, total urinary NNAL and exhaled CO in 30 subjects who were regular, exclusive (zero use of other tobacco/nicotine products during the prior 3 months) users of either second-generation (G2) (n = 9) or third-generation (G3) (n = 11) E(N)NDS products containing nicotine, or CC (n = 10).

64. Mean CO levels were significantly higher in smokers (13.9 ppm) compared with G2 (2.3 ppm) or G3 (3.4 ppm) users. There were significant differences between G2 and G3 users. Mean urinary total NNAL levels were also significantly higher in smokers (1.47 pmol/mL) than in G2 (0.17 pmol/mL) or G3 (0.21 pmol/mL) users, but not significantly different between G2 and G3 users. Total NNAL was below the LOD of 0.015 pmol/mL in 6 E(N)NDS users, but higher than expected in 7 users (0.099, 0.521, 0.326, 0.341, 0.169, 1.446, 0.2510 pmol/mL). Authors considered that this was most likely due to misreporting of lack of exposure to combustible tobacco during the previous 3 months in these subjects, but that contamination of E(N)NDS liquids with NNK at levels higher than those reported in the literature, or inter-

[33] Factors such as age, gender, proportion of family who were smokers (a full list is given in Table 2 of the publication).

individual variability in NNK metabolism, could not be ruled out as alternative or contributing explanations.

65. Bustamante et al. (2018) investigated the endogenous formation of the tobacco-specific oral and oesophageal NNN in E(N)NDS users. Salivary NNN, nornicotine, and nicotine as well as urinary tobacco biomarkers, including total NNN, were analysed in 20 E(N)NDS users, 20 CC smokers, and 19 nonsmokers. Nornicotine and NNN levels in E(N)NDS used by the study participants were also analysed. Mean NNN in saliva of E(N)NDS users was 14.6 (+/-23.1) pg/mL, ranging from below the LOQ to 76.0 pg/mL. In CC smokers, salivary NNN ranged from below LOQ to 739 pg/mL, with 80% of smokers having salivary NNN in the range of levels found in E(N)NDS users. Very low levels of urinary total NNN were present in 5 of 20 E(N)NDS users (ranging from 0.001 to 0.01 pmol/mL urine). Only trace levels of NNN were found in E(N)NDS liquids. Authors concluded that these findings demonstrated that NNN is formed endogenously in E(N)NDS users, but that the known carcinogenic potency of NNN warrants further investigations into the potential consequences of its endogenous formation. They recommended that salivary NNN, rather than urinary total NNN, which accounts for only 1-3% of the NNN dose, should be used to monitor exposure to this carcinogen in E(N)NDS users.

66. Serum levels of 42 elements were measured by ICP-MS in nonsmokers (n = 58), CC smokers (n = 58), and E(N)NDS users (n = 34) in Romania (Badea et al. 2018). CC smokers showed the highest levels of copper, molybdenum, zinc, antimony, and strontium. E(N)NDS users presented the highest concentrations of selenium, silver, and vanadium. Beryllium, europium and lanthanides were detected more frequently among E(N)NDS users (20.6%, 23.5%, and 14.7%) than in CC smokers (1.7%, 19.0%, and 12.1%) and the number of detected rare earth elements was also higher among E(N)NDS users (11.8% showed more than 10 different elements). Serum levels of cerium and erbium increased with longer duration of use of E(N)NDS. Authors concluded that CC smoking is mainly a source of heavy metals while the use E(N)NDS is a potential source of rare earth elements. However, they noted that these elements were detected at low concentrations.

67. Aherrera et al. (2017) reported some correlations between levels of nickel and chromium in E(N)NDS aerosols and in used E(N)NDS liquid (i.e. residing in the device after use) with levels in biospecimens (saliva, urine, exhaled breath) in a group of approximately 50 E(N)NDS users. Higher aerosol and tank nickel were associated with higher nickel and chromium levels in saliva. There was also a positive correlation of aerosol nickel concentration with urinary nickel levels. No correlations were observed between biomarker levels and levels of these metals in unused E(N)NDS liquids, leading the authors to conclude that the sources of these metals to which E(N)NDS users are exposed are derived from the heating coil, not the E(N)NDS liquid. Some self-reported parameters of E(N)NDS use also indicated correlation with Ni levels in E(N)NDS user biospecimens, such as shorter time to first E(N)NDS use after waking, more frequent changing of the heating coil, preferred voltage, and higher volume of E(N)NDS liquid used.

CC smokers vs. dual users

68. Two studies that compared urinary NNAL levels in dual CC/E(N)NDS users and CC-only users found that the NNAL levels were lower in the dual users. In one of these studies, the dual users smoked fewer CC per day than the CC-only users, but exhaled CO and urinary cotinine levels were not different between the 2 groups (Piper et al. 2018). In the study of Nollen et al. (2017), the dual users smoked more CC per day than CC-only users, but had lower levels of urinary nicotine, cotinine, and tobacco nicotine equivalents. Neither of these studies investigated an E(N)NDS-only group.

Risk assessment of potential user exposure to some commonly reported constituents of E(N)NDS aerosols

69. A study conducted by Dawkins et al. (2018) in the Southeast of England investigated 'vaping behaviour' in 20 subjects when using a tank-style E(N)NDS device ad libitum for periods of 1 week under each of 4 different conditions: fixed power with 6 mg/mL nicotine; variable power with a 6 mg/mL nicotine; fixed power with 18 mg/mL nicotine; variable power with 18 mg/mL nicotine. Four different flavours were available for choice. Various puff parameters were measured. Mean (SD) total numbers of puffs taken per day were reported as follows: 338 (161) using fixed power/6 mg/mL; 308 (135) using variable power/6 mg/mL; 279 (127) using fixed power/18 mg/mL; 272 (128) using variable power/18 mg/mL. The lowest (272 puffs/day) and highest (338 puffs/day) mean values are used for calculations made in the following sections.

70. Highest levels of particulate matter, PG, glycerol, nicotine, formaldehyde, acetaldehyde, and acrolein reported in the following sections are those identified from the studies evaluated in the preceding sections of this discussion paper.

Particulate matter

Exposure assessment

71. The highest reported values were:

- PNC: **1.50×10^{10} particles/cm^3** (Baassiri et al. 2017)
- TPM: 77.9 mg/15 puffs [equivalent to **5.19 mg/67 cm^3 puff** or **77,512 mg/m^3** in the aerosol] (Baassiri et al. 2017)

Regulations and guideline values

72. The World Health Organization (WHO) Air Quality Guideline (AQG) levels for $PM_{2.5}$ are **0.025 mg/m^3** (24-h mean) and **0.010 mg/m^3** (annual mean). Values for PM_{10} are **0.050 mg/m^3** (24-h mean) and **0.020 mg/m^3** (annual mean). The basis of the AQG is 'the lowest levels at which total, cardiopulmonary and lung cancer

mortality have been shown to increase with more than 95% confidence in response to long-term exposure to $PM_{2.5}$ (WHO 2006).

Propylene glycol

Exposure assessment

73. The highest reported levels of PG were **0.709 mg/55 cm^3 puff** [equivalent to a concentration of 12,890 mg/m^3 in the aerosol] (Margham et al. 2016) or 0.7 mg/35 cm^3 puff [equivalent to a concentration of **20,000 mg/m^3** in the aerosol] (Kienhuis et al. 2015).

74. Using the data from Margham et al. (2016) and a mean of 272–338 puffs/day from Dawkins et al. (2018), and assuming that 100% of the PG inhaled is absorbed, this would lead to a mean daily PG exposure of **193–240 mg/day**, or **2.76–3.43 mg/kg bw/day** for a 70 kg adult.

Regulations and guideline values

Inhalation

75. From discussions at the July 2018 COT meeting (TOX/2018/23), the COT established a health-based guidance value (HBGV) for continuous exposure to PG of **2.9 mg/m^3**. This was based on a lowest observed adverse effect level (LOAEL) of 160 mg/m^3 for nasal haemorrhaging from the study of Suber et al. (1989), in which rats were exposed to PG aerosol for 6 h/d, 5 d/wk, with adjustment for continuous exposure (x5.6) and using an uncertainty factor (UF) of 10 for inter-individual variation. For a 70 kg adult breathing 20 m^3 air per day, this would equate to an exposure of 0.83 mg/kg bw/day.

76. In the UK, the workplace exposure limits (WELs) (8 h time-weighted average (TWA)) for long-term exposure to PG are 150 ppm, or **474 mg/m^3**, for total vapour + particulates, and **10 mg/m^3** for particulates alone (HSE 2011). No short-terms WELs are available.

77. Other agencies have established HBGVs for PG based on the study of Suber et al. (1989) (see TOX/2018/23 for more details).

78. The Dutch Expert Committee on Occupational Standards established an 8-h TWA (vapour + aerosol) of **50 mg/m^3** based on a no observed adverse effect level (NOAEL) of 160 mg/m^3 for increased numbers of goblet cells. The Committee also recommended that health-based occupational exposure limits for inhalable and respirable dust should be applied to aerosols of PG (HCN 2007).

79. The German Committee on Indoor Guide Values recommended a health precaution guide value (RW I, guideline value I[34]) of **0.06 mg/m^3** for PG, based on a health hazard guide value (RW II, guideline value II) of 0.6 mg/m^3, derived using a LOAEL of 160 mg/m^3 for nasal haemorrhage (Umweltbundesamtes 2017).

80. The US Agency for Toxic Substances and Disease Registry (ATSDR) established an intermediate-duration minimum risk level (MRL) for PG of 0.009 ppm [**0.028 mg/m^3**], based on a LOAEL of 51 ppm [160 mg/m^3] for nasal haemorrhaging (ATSDR 1997).

Non-inhalation

81. The European Medicines Agency (EMA) specified a limit of **500 mg/kg bw/day** maximum daily dose for individuals aged 5 years and above, considered to be safe by any duration and route of administration, with the exception of inhalation (EMA 2014).

82. WHO set an acceptable daily intake (ADI) for oral intake of PG in food of **25 mg/kg bw/day**, citing a level causing no toxicological effect in the rat and in the dog of 2500 mg/kg bw (FAO/WHO 1974, FAO/WHO 2002).

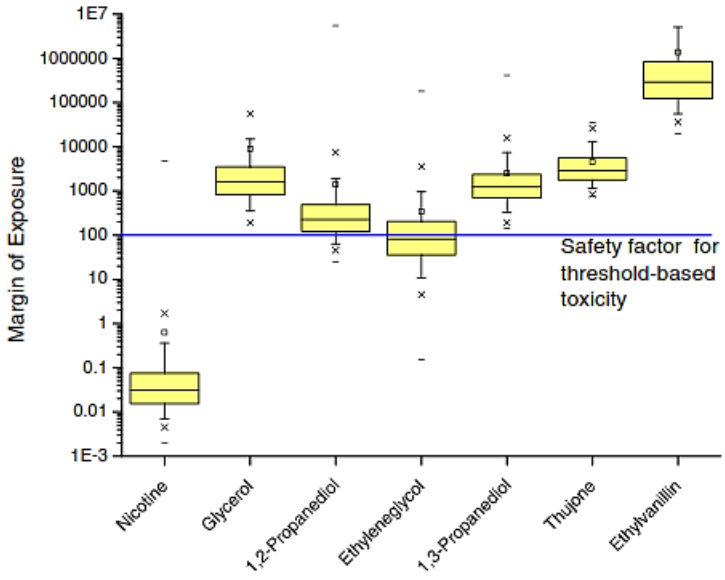
Published risk assessments of exposure to PG from E(N)NDS use

83. Hahn et al. (2014) performed a risk assessment of PG exposure from E(N)NDS, applying a 'margin of exposure' (MOE) approach. A total of 54 E(N)NDS liquid samples, including those labelled as nicotine-free or containing nicotine (range 6–54 mg/mL), were analysed by NMR spectroscopy for content of several chemicals, including PG, glycerol, and nicotine. Daily exposure was estimated from these analyses using probabilistic analysis, estimates of E(N)NDS liquid usage per day, vaporisation percentage, and a standard bodyweight of 73.9 kg. Estimated exposure for PG was calculated as a mean of 14.5 ± 12.4 mg/kg bw/day or a median of 11.0 mg/kg bw/day (P_5–P_{95}, 1.3–39.3). Toxicological threshold values (benchmark dose (BMD), or otherwise no observed effect level (NOEL), NOAEL, or LOAEL) were obtained from monographs of national or international risk assessment bodies. The JECFA/WHO ADI of 25 mg/kg bw/day, based on a NOAEL of 2500 mg/kg bw/day from 2 y studies in rats and dogs (no effects in rats, increased erythrocyte destruction in dogs) was chosen for the toxicological threshold. The authors

[34] RW I represents the concentration of a substance in indoor air for which, when considered individually, there is no evidence that life-long exposure would have an adverse health impact. RW II represents the concentration of a substance that, if reached or exceeded, requires immediate action as this concentration could pose a health hazard. It may be defined as a short-term value (RW II K) or a long-term value (RW II L). For more information, see: https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/german-committee-on-indoor-guide-values#textpart-3 (accessed 06/04/18).

considered that using a toxicological endpoint based on animal data, an MOE above 100 would be judged as acceptable, MOE < 100 would be judged as 'risk', and MOE < 10 judged to pose 'high risk'. MOE distributions (25th–75th and 5th–95th percentile distributions) were presented in Figure 3 of the publication, which is reproduced below in Figure 1. The distribution for PG was above 100 except for some instances above the 75th percentile. The authors considered that the risks from exposure of users to PG from E(N)NDS would be minor.

Figure 1. Margin of exposure (MOE) for compounds occurring in E(N)NDS based on probabilistic exposure estimation (simulation with 10,000 iterations). [reproduction of Figure 3 from the publication of Hahn et al. (2014)].
The box is determined by the 25th and 75th percentiles. The whiskers are determined by the 5th and 95th percentiles. 1st and 99th percentiles are marked by x, while minimum and maximum are marked with a dash. Values above 1E7 are not shown.



84. Kienhuis et al. (2015) published a risk assessment of PG exposure from E(N)NDS. Components of commercial, nicotine-free 'shisha pens' were evaluated and the major constituent was identified as a 54%/46% mixture of PG/glycerol, which the authors calculated to produce 0.7 mg/puff PG and 0.6 mg/puff glycerol. Based on a 50-70 mL puff volume, the maximum alveolar concentration of PG after 1 puff was estimated to be 430-603 mg/m^3. Referring to a study of human volunteers reported by Wieslander, Norback and Lindgren (2001), in which concentrations of PG in the range 176-851 mg/m^3 caused acute eye and upper airway irritation in a small proportion of individuals (thus considered as a LOAEL), Kienhuis and colleagues determined MOEs for PG in the range 0.3-2.0 [35]. In a published commentary on this study, Farsalinos and Baeyens (2016) criticised the approach of Kienhuis and

[35] i.e. using the LOAELs of 176-851 mg/m^3 as the range of PoD values, and alveolar PG concentrations from one 50-70 mL puff to be 430-603 mg/m^3

colleagues, specifically the use of throat irritation symptoms for the point of departure (PoD) (noting that 'throat hit' is in fact a desired effect for some E(N)NDS users) and the use of a PoD determined from 1-minute continuous exposure for calculation of an MOE for a single puff (which would have a duration in the range of 1 s).

Risk assessment

85. The estimated exposure (see paragraph 74) of 2.76-3.43 mg/kg bw/day is 3-4 times the inhalation HBGV of 0.83 mg/kg bw/day (2.9 mg/m^3) proposed by COT in July 2018.

Glycerol

Exposure assessment

86. The highest reported level of glycerol was **1.579 mg/55 cm^3 puff** [equivalent to a concentration of **28,709 mg/m^3** in the aerosol] (Margham et al. 2016).

87. Using the data from Margham et al. (2016) and a mean of 272–338 puffs/day from Dawkins et al. (2018), and assuming that 100% of the glycerol inhaled is absorbed, this would lead to a mean daily glycerol exposure of **429–533 mg/day**, or **6.13–7.61 mg/kg bw/day** for a 70 kg adult.

Regulations and guideline values

Inhalation

88. From discussions at the July 2018 COT meeting (TOX/2018/23), the COT established an HBGV for continuous exposure to glycerol of **11.8 mg/m^3**. This was based on a PoD of 662 mg/m^3 (NOAEL) from the rat inhalation study of Renne et al. (1992), in which rats were exposed to glycerol aerosol 6 h/d, 5 d/wk, for 13 wk, with adjustment for continuous exposure (x5.6) and applying a UF of 10 for inter-individual variation. For a 70 kg adult breathing 20 m^3 air per day, this would equate to an exposure of 3.37 mg/kg bw/day.

89. The long-term WEL for glycerol (glycerin) mist in the UK is **10 mg/m^3** TWA (HSE 2011). No short term WELs are available.

90. One other agency has established an HBGV for inhalation exposure to PG, based on the study of Renne et al. (1992) (see TOX/2018/23 for more details). DFG in Germany set a maximum workplace concentration (MAK value) of **200 mg/m^3**, based on a NOAEL of 662 mg/m^3 (Hartwig A 2017).

Non-inhalation

91. In an evaluation of the use of glycerol (E 422) as a food additive, the European Food Safety Authority (EFSA) concluded that toxicological studies in animals did not provide any indication for adverse effects, including at the highest

dose tested in a chronic toxicity study (10,000 mg/kg bw/day), and that there is no need for a numerical ADI for glycerol (E 422). However, it was noted that production methods may lead to the presence or formation of contaminants which are of toxicological concern (EFSA 2017).

Published risk assessments of exposure to glycerol from E(N)NDS use

92. Using the methodology presented in paragraph 83, Hahn et al. (2014) performed a risk assessment of glycerol exposure from E(N)NDS, applying an MOE approach. Estimated exposure for glycerol was calculated as a mean of 9.0 ± 8.9 mg/kg bw/day or a median of 6.2 mg/kg bw/day (P_5–P_{95}, 0.6–27.2). A NOAEL of 10,000 mg/kg bw/day based on no effects observed in a 2-y study in rats (OECD 2002) was selected for the toxicological threshold. The MOE distribution range for glycerol was above 100 (including at the 99th percentile).

93. In a preliminary risk assessment of glycerol exposure from E(N)NDS (methodology described in paragraph 84, above), based on a 50-70 mL puff volume, Kienhuis et al. (2015) estimated the maximum alveolar concentrations of glycerol after 1 puff to be 348-495 mg/m^3. An MOE for glycerol was not calculated due to the lack of study data on the effects of inhalation in humans. However, the authors noted that the estimated alveolar concentrations were in a similar range to the LOAEL of 662 mg/m^3 for local irritant effects in rats exposed continuously to glycerol for 13 weeks in the study of Renne (1992). Farsalinos and Baeyens (2016) criticised this approach, noting that the use of continuous exposure studies for risk assessment of glycerol or PG from E(N)NDS use (where an average puff may have a duration of around 1 s) is inappropriate and would probably overestimate any risk: they commented that this approach would be useful if evaluating total absorption for relation to any systemic effects, but would not be of value for local effects considering the important differences in exposure patterns.

Risk assessment

94. The estimated exposure (see para 87) of 6.13-7.61 mg/kg bw/day is approximately 2-fold the inhalation HBGV of 3.37 mg/kg bw/day (11.8 mg/m^3) proposed by COT in July 2018.

Nicotine

Exposure assessment

95. The highest reported level of nicotine was **93 µg/70 cm^3 puff** [equivalent to a concentration of **1329 mg/m^3** in the aerosol] from testing of 9 products containing nicotine at concentrations ranging from 11.5–27.4 mg/mL in ENDS liquid (Laugesen et al. 2015).

96. Using the data of Laugesen et al. (2015) and a mean of 272–338 puffs/day from Dawkins et al. (2018), and assuming that 100% of the nicotine inhaled is

absorbed, this would lead to a mean daily nicotine exposure of **25.3–31.4 mg/day**, or **0.361–0.449 mg/kg bw/day** for a 70 kg adult.

Intake of nicotine from CC smoking

97. Average daily intake of nicotine in 22 CC smokers was reported as 37.6 ± 17.7 mg, with a wide variation between subjects (10.5–78.6 mg). This was estimated from metabolic clearance data obtained after intravenous infusion of nicotine and from blood and urinary nicotine concentration data obtained over 24 h while the subjects were smoking CC. This would be equivalent to approximately 0.5 mg/kg bw/day for a 70 kg adult. Average nicotine intake per CC was 1.04 ± 0.36 mg (Benowitz and Jacob 1984).

Regulations and guideline values

Inhalation

98. The UK WEL for nicotine is **0.5 mg/m^3** 8 h TWA, with a 15 min short term exposure limit (15 min short-term exposure limit (STEL)) of **1.5 mg/m^3** (HSE 2018). Workplace exposure limits in many other EU countries are also **0.5 mg/m^3** 8 h TWA, except for Sweden (**0.1 mg/m^3** 8 h TWA)[36]. The National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL) and Occupational Safety and Health Administration (OSHA) recommended permissible exposure limit (PEL) values for nicotine are **0.5 mg/m^3** TWA [skin][37]. The NIOSH IDLH (immediately dangerous to life or health) is **5 mg/m^3**, based on a fatal human oral dose estimated as 50 to 60 mg[38].

99. The United States Environmental Protection Agency (US EPA) evaluated occupational risk of short- and intermediate-term use of nicotine as a pesticide (in the format of smoke-generating canisters), by certified applicators, on ornamental plants in greenhouses (only) for a reregistration application eligibility decision. A **NOAEL of 1.25 mg/kg bw/day** was identified for hepatotoxicity in a 10-day rat drinking-water study (Yuen et al. 1995) (EPA 2008). The Agency described using an MOE approach. The Agency determined that an **MOE of 1000** would be considered to be protective of human health (10 for inter-species extrapolation, 10 for intra-species variability and 10 for database uncertainty). The major potential source of risk for exposure was considered to be by inhalation, with relative smaller exposure dermally.

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[36] See http://limitvalue.ifa.dguv.de/, accessed 28/02/2019.
[37] The " [skin] " designation indicates the potential for dermal absorption; skin exposure should be prevented as necessary through the use of good work practices, gloves, coveralls, goggles, and other appropriate equipment.
[38] See https://www.cdc.gov/niosh/idlh/54115.html, accessed 28/02/2019.

Non-inhalation

100. EFSA set an oral acute reference dose (ARfD) of **0.0008 mg/kg/bw/day** for nicotine (EFSA 2009). This value was based on the study of Lindgren et al. (1999) (cited in EFSA 2009), in which a dose-response relationship for electroencephalographic parameters and heart-rate frequency over a range of nicotine doses (i.v. infusion) were evaluated in 14 regular CC smokers. From these data, EFSA determined a LOAEL of 0.0035 mg/kg bw/day for pharmacological effects (slight, transient and rapidly reversible increase of the heart rate in humans). The ARfD was determined using an overall UF of 10[39] and a correction factor of 0.44 for oral bioavailability of nicotine (extrapolation from i.v to oral route[40]). Given that nicotine has a short biological half-life and does not accumulate in the body, and that the most sensitive effect was considered to be the pharmacological effect on the cardiovascular system, EFSA considered that the value set for the ARfD would be suitable to protect from chronic effects and could also be applied as the ADI.

101. The report of EFSA (2009) noted that in 2009, the German Federal Institute for Risk Assessment (BfR) also established an ARfD for nicotine of **0.0008 mg/kg bw/day**, based on the study of Lindgren et al. (1999). A LOAEL of 0.0035 mg/kg bw/day was selected as the PoD, based on increased heart rate. A safety factor of 10 for intra-species variability, and a correction of 0.44 for oral bioavailability was used to derive the ARfD (data cited in EFSA 2009).

102. EFSA (2009) also noted that in an assessment of nicotine under the EU peer review process for pesticides, in 2007, a UK Rapporteur proposed an ARfD and ADI of **0.0001 mg/kg bw/day**. This was based on data reported by Woolf et al. (1997) (cited in EFSA 2009). Woolf carried out a post-marketing surveillance study of data collected at US poison centres, including 36 children aged 0–15 y exposed to transdermal nicotine patches (by either dermal or oral route). Clinical signs of toxicity were reported at approximately 0.03–0.8 mg/kg/bw day. The lowest estimated systemic exposures of nicotine associated with adverse effects were reported to be

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[39] EFSA noted that “The LOAEL is considered to be close to the NOAEL and the overall UF of 10 would be sufficient to cover not only human variability but the extrapolation from the LOAEL to NOAEL for the pharmacological effect observed at the LOAEL.”

[40] For comparison, in a report of ‘Metabolism and Disposition Kinetics of Nicotine’, Hukkanen et al. (2005) report % bioavailability for nicotine administered as single doses by various routes as follows: Smoking 1 CC (80-90%); i.v. approx 5.1 mg (100%); Nasal spray 1 mg (60-80%); Gum 2-4 mg (55-78%; Inhaler 4 mg (51-56%); Lozenge 2-4 mg (50-79%); Transdermal patch 14-21 mg/24 h (68-100%); s.c. injection 2.4 mg (100%); Oral capsule 3-4 mg (44%); Oral solution approx. 3 mg (20%); Enema approx. 3.5 mg (15-25%). See https://pdfs.semanticscholar.org/9043/b736c593390f4389409f8051c95b75e1de97.pdf (accessed 04/03/2019).

< 0.01 mg/kg bw/day. The value of 0.01 mg/kg bw/day was taken as a lowest observed effect level (LOEL). The ARfD was calculated using an UF of 100 (10 for intra-species variability and 10 for use of a limited data set) (UK DAR 2007, data cited in EFSA 2009).

103. Finally, the report of EFSA (2009) also noted that the French Food Safety Agency (Agence Française de Sécurité Sanitaire des Aliments, AFSSA) had prepared a report providing scientific and technical advice concerning mushroom contamination by nicotine. AFSSA endorsed the ADI and ARfD of **0.0001 mg/kg bw/day** proposed by the UK in 2007 (information cited in EFSA 2009).

Published risk assessments of exposure to nicotine from E(N)NDS use

104. Using the methodology presented in paragraph 83, Hahn et al. (2014) performed a risk assessment of glycerol exposure from E(N)NDS, applying an MOE approach performed a risk assessment of nicotine exposure from E(N)NDS, applying an MOE approach. Estimated exposure for nicotine was calculated as a mean of 0.38 ± 0.39 mg/kg bw/day or a median of 0.25 mg/kg bw/day (P_5–P_{95}, 0.02–1.15). The EFSA ADI of 0.0008 mg/kg bw/day, based on a LOAEL of 0.008 mg/kg bw/day for heart-rate acceleration in humans was chosen for selection of the toxicological threshold. MOE distributions (25^{th}–75^{th} and 5^{th}–95^{th} percentile distributions) were presented in Figure 3 of the publication, which is reproduced in Figure 1, above). The MOE distribution for nicotine was entirely below 10, and mostly below 0.1 (including 25^{th} percentile exposure and above).

105. Nicotine was considered by the authors to be the compound with the highest risk in E(N)NDS liquids. Authors also noted that nicotine exposure would exceed the EFSA ADI of 0.0008 mg/kg bw/day for nicotine residues in food products. In discussing the suitability of the EFSA endpoint of heart-rate acceleration as an (adverse) endpoint for risk assessment of nicotine, the authors noted that data on the potential chronic risk of nicotine exposure were not available and thus they concurred with EFSA that application of heart-rate acceleration as the most sensitive human endpoint is appropriate until better data become available. This would be particularly applicable to non-(nicotine)-tolerant users of E(N)NDS, and they considered that such users should be warned against using E(N)NDS products with higher nicotine contents. It was also noted that the potential hazard of nicotine dependence, which is a function of form and speed of nicotine delivery, could not be considered due to lack of adequate dose–response data. Finally, the authors commented that as some products marketed as not containing nicotine did actually contain nicotine, this could expose consumers to the hazard of developing nicotine dependence from a product purchased as containing no nicotine (Hahn et al. 2014).

106. Baumung et al. (2016) used an MOE approach for risk assessment of tobacco smoke constituents, including nicotine. Data on nicotine exposure from CC were gathered from 3 sources: a point estimate of 0.229 mg/kg bw/day for a 70 kg person

smoking 20 CC[41] per day (Xie et al. 2012); a probabilistic value of 0.359 mg/kg bw/day for a person of average weight of 73.9 ± 12 kg using data from the 2006 China Health and Nutrition Survey (average 16.4 CC[42] per day, P_5–P_{95}, 3–30 CC/day) (Lachenmeier and Rehm 2015); and a probabilistic value of 0.543 mg/kg bw/day for a person of average weight 63.3 kg (P_5–P_{95}, 46.7–84.2 kg), smoking 10–20 CC[43]/day (Cunningham et al. 2011). Toxicological thresholds for nicotine were identified from the literature and, additionally, $BMDL_{10}$ values were calculated by the authors based on data from key studies noted in published risk assessments. These data are summarised in Table 7, below.

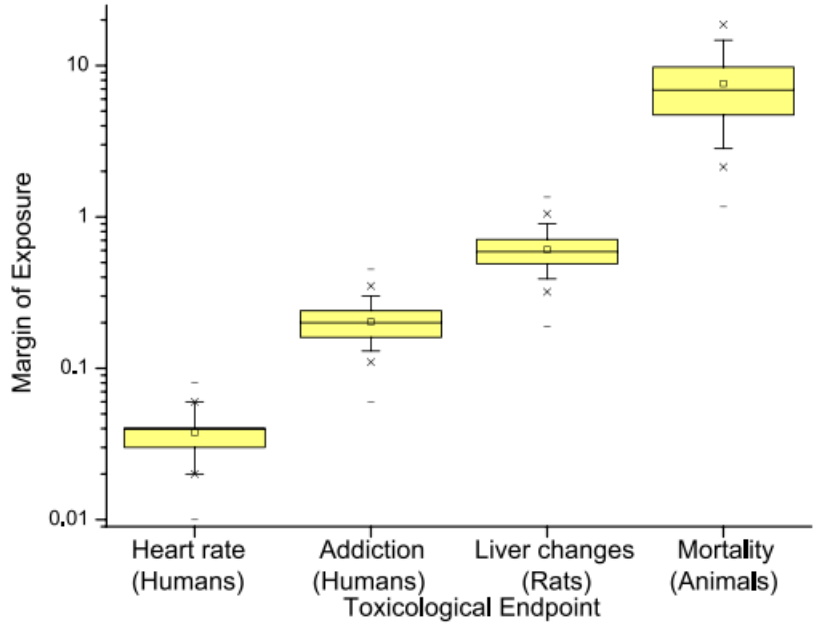
Table 7. Toxicological thresholds for nicotine. Reproduction of Table 1 from Baumung et al. (2016).

Species	Effect	Type of endpoint	Value (mg/kg bw)
Humans, i.v. acute	Heart rate acceleration	LOAEL	0.008
Humans, i.v. acute	Heart rate acceleration	BMDL for BMR=ISD	0.013
Humans, chronic, cigarette use	Addiction	Threshold	0.07
Humans (children), dermal, acute	Various symptoms of intoxication	LOAEL	0.01
Humans (children), dermal, acute	Various symptoms of intoxication	BMDL10	0.004
Various animal species, acute	Mortality (LD50) study	BMDL10	3
Rats, 10 day study	Liver; fatty change	BMDL10	0.27
Rats, 10 day study	Liver; focal necrosis	BMDL10	0.24
Rats, 10 day study	Liver; dark cell change	BMDL10	0.21
Rats, 10 day study	Pathological changes in liver	NOAEL	1.25

[41] Constituent yield from 1R4F cigarettes under the Health Canada Intense machine-smoke regime [note: the 1R4F reference cigarette contains 0.8 mg].
[42] Analyses of 30 brands of cigarettes sold in China using the Canadian intense smoking regime (2.09 ± 0.25 mg nicotine/cigarette).
[43] 1.65–1.89 mg nicotine/cigarette.

107. Over the range of 4 different toxicological endpoints and species used, calculated MOE values for nicotine ranged from 0.04–7.6. Data were summarised in Figure 1 of the publication by Baumung et al. (2016), which is reproduced in Figure 2, below. The average MOE for 'changes in rat liver' (fatty change, focal necrosis and dark cell change) was 0.61. The authors considered that this MOE may underestimate the risk of nicotine due to the very short duration of the liver toxicity study (10 days). The authors also calculated MOEs for other CC smoke constituents, and they noted that MOEs for nicotine were in the same dimension as those for CC smoke toxicants with the lowest MOEs, such as acrolein, formaldehyde, and cadmium compounds.

Figure 2. Margin of Exposure for nicotine daily smokers considering different toxicological endpoints (reproduction of Figure 1 from Baumung et al. 2016). Boxes represent 25th and 75th percentiles, whiskers the 5th and 95th percentiles and x the 1st and 99th percentiles, and - the minimum and maximum values.



Risk assessment

108. The estimated daily exposure from 272–338 ENDS puffs of 0.361–0.449 mg/kg bw/day nicotine (see para 96) is within the same range as the average daily exposure of 0.5 mg/kg bw/day nicotine estimated from CC smoking (see paragraph 97).

109. The estimated exposure of 0.361-0.449 mg/kg bw/day nicotine from ENDS is approximately 300-fold the level that was considered by US EPA to be protective for human health for use in pesticide spraying (see paragraph 99) and 500-fold the ARfD and ADI values of 0.0008 mg/kg bw/day established by EFSA for intake of nicotine from food (see paragraph 100).

Formaldehyde

Exposure assessment

110. The highest reported level of formaldehyde was **97 µg/50 cm^3 puff** [equivalent to a concentration of **1940 mg/m^3** in the aerosol] (Sleiman et al. 2016).

111. Using the data from Sleiman et al. (2016) and a mean of 272–338 puffs/day from Dawkins et al. (2018), and assuming that 100% of the formaldehyde inhaled is absorbed, this would lead to a mean daily formaldehyde exposure of **26–33 mg/day**, or **0.38–0.49 mg/kg bw/day** for a 70 kg adult.

Regulations and guideline values (inhalation)

112. Workplace exposure limits for formaldehyde in the UK are **2.5 mg/m^3** for both long-term (8 h TWA) and short-term (15 min STEL) exposures (HSE 2018). The European Commission Scientific Committee on Occupational Exposure Limits (SCOEL) recommend a Limit Value of 0.3 ppm [**0.37 mg/m^3**] (8 h TWA) with a STEL of 0.6 ppm [**0.74 mg/m^3**] (SCOEL 2016). Workplace exposure limits reported for 16 individual EU countries, as summarised by SCOEL (2016), were in the range of **0.15–2.5 mg/m^3** (8 h TWA) and **0.37–2.5 mg/m^3** (15 min STEL).

113. The WHO short-term (30 min average concentration) guideline for formaldehyde in indoor air is **0.1 mg/m^3**, for prevention of sensory irritation in the general population. This is based on a NOAEL of 0.63 mg/m^3 for sensory irritation (conjunctival redness and eye blinking) in humans, with adjustment factor of 5 derived from standard deviation of sensory irritation threshold. Evaluations for protection of health for long-term effects were based on a NOAEL of 1.25 mg/m^3 for cell proliferation (limited to site of contact) from studies in rats. Based on this, two different approaches that were used established guideline values for cancer effects of approximately 0.2 mg/m^3. Thus, WHO considered that the short-term (30 min) guideline of 0.1 mg/m^3 would also prevent long-term health effects, including cancer (WHO 2010).

114. The ATSDR acute inhalation MRL for formaldehyde is 0.04 ppm [**0.05 mg/m^3**]. This is based on a minimal LOAEL of 0.4 ppm for nasal and eye irritation in humans, and using an UF of 10 (rounded) (3 for use of a minimal LOAEL and 3 for human variability) (ATSDR 1999). The intermediate-duration inhalation MRL is 0.03 ppm [**0.037 mg/m^3**], based on a NOAEL of 0.98 ppm for nasopharyngeal irritation in cynomolgous monkeys exposed for 22 h/d, 7 d/wk, for 26 wk, using an UF of 30 (3 for inter-species extrapolation and 10 for human variability) (ATSDR 1999). The chronic inhalation MRL is 0.008 ppm [**0.01 mg/m^3**). This is based on clinical symptoms of mild irritation of the eyes and upper respiratory tract and mild damage to the nasal epithelium observed in workers exposed for 10.4 y (range, 1–36 y) to an average TWA concentration of 0.24 ppm (range, 0.04–0.4 ppm). Such effects were considered to be a minimal LOAEL. The chronic MRL was determined by using a UF of (3 for use of a LOAEL and 10 for human variability)

(ATSDR 1999). For a 70 kg adult breathing 20 m^3 air per day, the ATSDR acute, intermediate, and chronic inhalation MRLs for formaldehyde would equate to exposures of 0.014, 0.011, and 0.003 mg/kg bw/day, respectively.

115. The US EPA quantitative estimate of carcinogenic risk from inhalation exposure to formaldehyde is **1.3 x 10^{-5} per µg/m^3** (EPA 1989).

Risk assessment

116. The estimated exposure (see para 111) of 0.38-0.49 mg/kg bw/day is approximately 30-fold the ATSDR acute inhalation MRL for formaldehyde of 0.014 mg/kg bw/day (0.05 mg/m^3), 40-fold the intermediate inhalation MRL of 0.011 mg/kg bw/day (0.037 mg/m^3), and 145-fold the chronic inhalation MRL of 0.003 mg/kg bw/day (0.01 mg/m^3) (see para 114).

Acetaldehyde

Exposure assessment

117. The highest reported level of acetaldehyde was **50 µg/50 cm^3 puff** [equivalent to a concentration of **1000 mg/m^3** in the aerosol] (Sleiman et al. 2016).

118. Using the data from Sleiman et al. (2016) and a mean of 272–338 puffs/day from Dawkins et al. (2018), and assuming that 100% of the acetaldehyde inhaled is absorbed, this would lead to a mean daily acetaldehyde exposure of **13.6–16.9 mg/day**, or **0.19–0.24 mg/kg bw/day** for a 70 kg adult.

Regulations and guideline values (inhalation)

119. Workplace exposure limits for acetaldehyde in the UK are **37 mg/m^3** for long-term exposure (8 h TWA) and **92 mg/m^3** for short-term exposure (15 min STEL) (HSE 2018).

120. Health Canada derived a tolerable concentration (TC) of **0.39 mg/m^3** for acetaldehyde, based on a $BMCL_{05}$ of 218 mg/m^3 for nasal olfactory lesions in rats exposed for 6 h/d, 5 d/wk, 4 wk, and applying a UF of 100 (10 for inter-species and 10 for intra-species variation) (Health-Canada 2000a). For a 70 kg adult breathing 20 m^3 air per day, this would equate to an exposure of 0.11 mg/kg bw/day. Health Canada calculated a tumorigenic concentration with 5% response (TC_{05}) of 86 mg/m^3 with a lower 95% confidence limit (TCL_{05}) of 28 mg/m^3 for inhalation of acetaldehyde (Health-Canada 2000a).

121. The US EPA reference concentration (RfC) for inhalation exposure to acetaldehyde is **0.009 mg/m^3**, based on the same study as used by Health Canada. The PoD used by EPA was a NOAEL of 273 mg/m^3 (adjusted for continuous exposure, 48.75 mg/m^3; human equivalent concentration (HEC), 8.7 mg/m^3) based on degeneration of the olfactory epithelium in rats exposed for 6 h/d, 5 d/wk, 4 wk. The RfC was calculated by applying a UF of 1000 (10 for inter-species and 10 for

intra-species variation, and 10 for extrapolation to chronic exposure) to the NOAEL (HEC) (EPA 1991). For a 70 kg adult breathing 20 m^3 air per day, this would equate to an exposure of 0.003 mg/kg bw/day. The EPA quantitative estimate of carcinogenic risk from inhalation exposure to acetaldehyde is 2.2 x 10^{-6} per µg/m^3 (EPA 1988).

Risk assessment

122. The estimated exposure (see para 118) of 0.19-0.24 mg/kg bw/day is approximately 2-fold the Health Canada TC of 0.11 mg/kg bw/day (0.39 mg/m^3) (see para 120) and 70-fold the US EPA RfC of 0.003 mg/kg bw/day (0.009 mg/m^3) (see para 121).

Acrolein

Exposure assessment

123. The highest reported level of acrolein was **21.5 µg/50 cm^3** puff [equivalent to a concentration of **420 mg/m^3** in the aerosol] (Sleiman et al. 2016).

124. Using the data from Sleiman et al. (2016) and a mean of 272–338 puffs/day from Dawkins et al. (2018), and assuming that 100% of the acrolein inhaled is absorbed, this would lead to a mean daily acrolein exposure of **5.85–7.27 mg/day**, or **0.084–0.100 mg/kg bw/day** for a 70 kg adult.

Regulations and guideline values (inhalation)

125. Workplace exposure limits for acrolein in the UK are **0.05 mg/m^3** for long-term exposure (8 h TWA) and **0.12 mg/m^3** for short-term exposure (15 min STEL) (HSE 2018).

126. WHO derived a TC of **0.0004 mg/m^3**, on the basis of a BMC_{05} of 0.14 mg/m^3 for non-neoplastic lesions in the nasal respiratory epithelium in rats exposed for 6 h/d, 3 d, and applying a UF of 100 (10 for inter-species variation and 10 for intra-species variation) (WHO 2002). For a 70 kg adult breathing 20 m^3 air per day, this would equate to an exposure of 0.0001 mg/kg bw/day.

127. Health Canada also derived a TC of **0.0004 mg/m^3**, on the same basis as used by WHO (2002) (Health-Canada 2000b). For a 70 kg adult breathing 20 m^3 air per day, this would equate to an exposure of 0.0001 mg/kg bw/day.

128. The US EPA RfC for inhalation exposure is **0.00002 mg/m^3**, based on a LOAEL of 0.4 ppm [0.9 mg/m^3] (adjusted for continuous exposure, 0.16 mg/m^3; HEC, 0.02 mg/m^3) for nasal lesions in rats exposed for 6 h/d, 5 d/wk, 13 wk. The RfC was calculated by applying a UF of 1000 (approximately 3 for inter-species extrapolation, 10 for intra-species variation, 10 for extrapolation to chronic exposure, and approximately 3 for use of a minimal LOAEL) (EPA 2003). For a 70 kg adult

breathing 20 m^3 air per day, this would equate to an exposure of 0.000006 mg/kg bw/day.

Risk assessment

129. The estimated exposure (see para 125) of 0.084–0.100 mg/kg bw/day is approximately 1000 times the WHO and Health Canada TC of 0.0001 mg/kg bw/day (0.0004 mg/m^3) (see paras 127 and 128) and 15000 times the US EPA RfC of 0.000006 mg/kg bw/day (0.00002 mg/m^3) (see para 129).

Summary

130. This report summarises data on substances to which users may be exposed via direct inhalation of E(N)NDS aerosol. The literature base comprised 3 previous COT discussion papers on E(N)NDS, namely TOX/2017/49 (particulate matter), TOX/2018/15 (metals), and TOX/2018/16 (which included collated publications on E(N)NDS liquid and aerosol constituents).

131. Data are summarised on concentrations of chemicals/species present in E(N)NDS aerosols from studies in which aerosol was produced directly via machine puffing. How well machine puffing actually represents real-life E(N)NDS use is not clear. In addition, as noted in previous COT discussion papers on E(N)NDS, study setups are variable, and levels measured may show a wide range between, and sometimes within, studies.

132. Particulate matter, PG, glycerol, nicotine, formaldehyde, acetaldehyde, and acrolein were detected in E(N)NDS aerosols in a number of studies. For all of these constituents, highest reported levels measured directly in E(N)NDS aerosols exceeded the regulatory or guideline levels in air that were identified. However, guideline levels generally relate to exposure patterns (e.g. continuous exposure, 8 h TWA, 15 min STEL) that are different to those that would be expected from E(N)NDS use (puffs of a few seconds duration inhaled intermittently throughout the day). Additionally, some commentators consider that usage conditions leading to the emission of aldehydes (from thermal breakdown of PG and/or glycerol) are unlikely to occur during ‘real-life’ E(N)NDS use, as this would require unpleasant ‘dry-puffing’, which users would avoid. For the purposes of comparisons in the risk assessments carried out here, estimated total daily intake for each chemical (mg/kg bw/day) was calculated from the highest reported level in 1 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 (mg/kg bw/day), either calculated from inhalation HBGVs in mg/m^3 if available, or as reported for other routes of exposure. In all instances these exposure was above the identified HBGVs.

133. A brief search was also performed to identify literature on levels of biomarkers of exposure to tobacco-related toxicants associated with E(N)NDS use, in comparison with levels in users of other tobacco products and in nonusers of tobacco products. Overall, around 20 studies of relevance were identified, including

short-term clinical studies and cross-sectional epidemiological studies. The majority of these reports noted that exposures to tobacco-related toxicants are lower from E(N)NDS use than from CC smoking, although some exposures may still be higher than levels measured in nonusers of tobacco products. Levels of nicotine and related metabolites associated with E(N)NDS use were generally reported to be either lower than or equivalent to those from CC smoking.

Questions for the Committee

134. Members are invited to comment on the information provided in this paper and to consider the following questions:

i. Do Members consider that the data presented indicate any specific chemicals may be of particular concern in relation to user exposure from E(N)NDS?

ii. Is the Committee able to draw any conclusions from the data presented on potential health risks associated with exposure of users to E(N)NDS aerosols?

iii. Are there any particular aspects of this paper that should be captured when a COT statement on E(N)NDS is prepared?

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat
February 2019

Abbreviations

1-OHP	1-hydroxypyrene
1-HOP	Metabolite of pyrene
3-HC	3-Hydroxycotinine
2-HPMA	2-hydroxypropylmercapturic acid
3-HPMA	3-hydroxypropylmercapturic acid
2-MHA	2-Methylhippuric acid
AAMA	N-acetyl-S-(2-carbamoylethyl)cysteine
ADI	Acceptable daily intake
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
AQG	Air quality guideline
ARfD	Acute reference dose
ATSDR	US Agency for Toxic Substances and Disease Registry
BCEP	Bis(2-chloroethyl) phosphate
BDCPP	Bis(1,3-dichloro-2-propyl) phosphate
BMC	Benchmark concentration
BMC05	Benchmark concentration, 5% response
BMCL05	Lower 95% confidence limit on the concentration associated with a 5% response
BMD	Benchmark dose
CEMA	2-Cyanoethylmercapturic acid
CC	Conventional cigarette
CO	Carbon monoxide
DBUP	Dibutylphosphate
DEHP	Diethylhexyl phthalate
DEP	Diethyl phthalate
DPhP	Diphenyl phosphate
EFSA	European Food Safety Authority
EMA	European Medicines Agency
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
EPA	US Environmental Protection Agency
HBGV	Health-based guidance value
HEC	Human equivalent concentration
HEMA	2-Hydroxyethyl mercapturic acid
HMPMA	3-Hydroxy-1-ethylpropylmercapturic acid
IDLH	Immediately dangerous to life or health
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
LOD	Limit of detection
LOQ	Limit of quantitation
MOE	Margin of exposure
MHBMA	Monohydroxy-3-butenyl mercapturic acid
MRL	Minimum risk level
NAB	N-nitrosoanabasine

NAT	N′-nitrosoanatabine
NG	Nicotine gum
NIOSH	US National Institute for Occupational Safety and Health
NO	Nitric oxide
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NNN	N-Nitrosonornicotine
NNK	4-(methylnitrosamino)-1–(3-pyridyl)-1-butanone
NNAL	4-(methylnitrosamino)-1–(3-pyridyl)-1-butanol
NRT	nicotine replacement therapy
OSHA	Occupational Safety and Health Administration
PAH	Polycyclic aromatic hydrocarbon
PATH	Population Assessment of Tobacco and Health
PEL	permissible exposure limit
PFR	Phosphate-based flame retardants
PG	Propylene glycol
PM2.5	Particulate matter 2.5 µm or less in diameter
PM10	Particulate matter 10 µm or less in diameter
PNC	Particle number concentration
PoD	Point of departure
REL	Recommended exposure level
RfC	Reference concentration
S-PMA	S-Phenylmercapturic acid
SCOEL	European Commission Scientific Committee on Occupational Exposure Limits
SLT or ST	Smokeless tobacco
STEL	Short-term exposure limit
TA	Total alkaloids
TC	Tolerable concentration
TC05	Tumorigenic concentration with 5% response
TCEP	Tris(2-chloroethyl) phosphate
TCL05	Lower 95% confidence limit of the TC05
TPM	Total particulate mass
TSNA	Tobacco-specific nitrosamine
TWA	Time-weighted average
TQD	Target quit date
UF	Uncertainty factor
VG	Vegetable glycerine (glycerol)
VOC	Volatile organic compound
WEL	Workplace exposure limit
WHO	World Health Organization

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 11: User exposure.

Details of Literature search carried out by NCET at WRc/IEH-C

A brief literature search was performed on 03/04/2019 to identify data on levels of biomarkers of exposure to tobacco-related toxicants associated with E(N)NDS use, in comparison with levels in users of other tobacco products and in nonusers of tobacco products.

This literature search was carried out in the PubMed database on 03/04/2019, using combinations of the search terms, ‘electronic cigarette’, ‘e-cigarette’, and ‘biomarker’, with no other search limitations. A total of 81 citations were identified from this search. In addition, the search was expanded to view ‘similar articles’ in the case of some of the relevant citations identified in primary search. Twenty-four publications of relevance to biomarkers of exposure to E(N)NDS were noted.