TOX/2018/24

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 4: Toxicological and epidemiological evaluations of E(N)NDS aerosol exposures.

1 Background

1. The COT is reviewing the potential human health effects of electronic nicotine delivery systems and electronic non-nicotine delivery systems (E(N)NDS). As part of this review, papers on characterisation of the aerosol particle fraction (TOX/2017/49) and exposure to metals from E(N)NDS use (TOX/2018/15) were discussed at the COT meetings in December 2017 and March 2018, respectively. A summary of publications describing the chemical constituents of E(N)NDS liquids and aerosols (excluding metals and flavourings) (TOX/2018/16) was also presented at the COT meeting in March 2018. A review of published data on the toxicity of the major constituents of E(N)NDS liquids, propylene glycol (PG) and glycerol (vegetable glycerine, VG) (TOX/2018/19) was presented at the COT meeting in May 2018. This present paper reviews published data on the toxicity of E(N)NDS aerosols.

2 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 (Bansal and Kim 2016). 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 a light-emitting diode (LED) light. Commercially available devices are sometimes categorised as first, second, or third generation. First-generation devices look like conventional cigarettes (CCs) 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[1].

3. Constituents that have been identified in E(N)NDS liquids and/or aerosols include PG, 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. The principal constituents, often in the range of 90-95% of the mass, are usually the solvents, PG and/or VG, which can be present in ratios ranging from 0:100 to 100:0 (see TOX/2018/16). The following sections summarise data relevant to the toxicity of E(N)NDS aerosol mixtures, including human epidemiological and clinical data and experimental studies in animals. Data from *in vitro* investigations have not been reviewed.

3 Search strategies

4. The following search strategies were combined to identify literature relevant to the toxicity of E(N)NDS aerosols: 1. Scopus and PubMed databases were searched using combinations of terms as described in Annex A. 2. Reports from authoritative bodies that have reviewed the toxicity and human health effects of exposure to E(N)NDS aerosols were appraised and relevant literature cited within these reports was identified. 3. Reference lists within the literature citations identified from 1 and 2, above, were inspected for further relevant literature.

4 Acute toxicity

4.1 Humans

5. Studies of the acute effects of exposure to E(N)NDS aerosols in humans have focussed on two main areas: parameters of respiratory function and cardiovascular indices.

4.1.1 Respiratory system

4.1.1.1 Acute effects on respiratory function

6. Experimental studies have evaluated the acute effects of E(N)NDS exposure on exhaled breath measurements and pulmonary function tests.

7. Healthy adult smokers (male and female) used a nicotine-containing E(N)NDS device (experimental group, n=30), or an equivalent device with the cartridge removed (control group, n=10; a sub-group randomly selected from the experimental group), *ad lib* for 5 min. The test device was NOBACCO MLB-MED[2] (Greece),

[1] see, http://ecigclopedia.com/the-4-generations-of-electronic-cigarettes/ (accessed 18/12/17)
[2] Constituents: >60% PG, <5% linalool, <10% nicotine, <5% tobacco essence, <1% methyl vanillin.

containing 11 mg/mL nicotine. On spirometry and impulse oscillometry testing, E(N)NDS aerosol exposure in the experimental group was associated with a significant 18% increase in peripheral flow resistance and 16% decrease in exhaled nitric oxide (NO) compared with baseline values, while significant changes were not observed after control tests without cartridge (Vardavas et al. 2012).

8. Ferrari et al. (2015) reported that 5 min *ad lib* use of a nicotine-free E(N)NDS device ('ELIPS C Series (Italy) with a 'Natur Smoke aroma Nocciola Antistress, 0 mg/mL nicotina' cartridge[3]) had no effect on lung function in 10 non-CC smokers. In addition, only minor effects were observed in 10 CC smokers: a small decline in forced expiratory flow at 25% of forced vital capacity (FVC) (FEF_{25}) and forced expiratory volume in 1 s (FEV_1) compared with baseline values. Subjects were healthy adult males and females.

9. Lappas et al. (2018) reported that acute E(N)NDS exposure affected parameters of respiratory mechanics and inflammation to a greater extent in asthmatic compared with non-asthmatic individuals, all of whom were regular dual users of CC and E(N)NDS. The test product was described as a 'new generation e-cigarette' (adjustable voltage) with 12 mg/mL nicotine[4]. Exposures were 10 puffs (4 s at 3.7 V, 1.6 Ω) at a 30-s puff interval, with (termed by authors, 'experimental') or without (termed by authors, 'control') the e-liquid and resistor coil present. Measurements were made before (termed by authors, 'screening'), and then immediately after (T0), 15 min after (T15), and 30 min after (T30) test exposures. Participants were adult male and female dual CC/E(N)NDS users, either non-atopic (n=27) or with mild asthma (n=27), and had abstained from using CC or E(N)NDS for 12 h prior to evaluations.

10. Parameters of spirometry were measured at baseline only. Asthmatics showed lower values than non-asthmatics for all spirometric parameters (FEV_1, FEV_1/FVC, peak expiratory flow (PEF), $FEF_{25-75\%}$, FEF_{25}, $FEF_{50\%}$, $FEF_{75\%}$) except FVC.

11. Impulse oscillometry system (IOS) measurements were made to evaluate the effects of test exposures on respiratory parameters, including total impedance at 5 Hz (Z5), resistance at 5 Hz (R5), 10 Hz (R10) and 20 Hz (R20), resonant frequency (f_{res}), reactance area (AX), reactance at 5 Hz (X5) and 20 Hz (X20). At 'screening', Z5, R5, R10, and f_{res} were higher in asthmatics compared with non-asthmatics prior to both 'control' and 'experimental' exposures. R20 was higher in asthmatics than non-asthmatics only in measurements made before 'experimental' exposures. Differences between asthmatics and non-asthmatics for X20 (lower in asthmatics) and AX (higher in asthmatics) were only observed for measurements made prior to 'control' exposures. After 'control'[5] exposures, there were no significant

[3] Constituents: >50% glycerin, 5-10% isotonic solution, 1-5% magnesium chloride, 0.1-1% natural flavour, 0.1-1% vitamin B12
[4] Constituents: 46.1% PG, 34.3% glycerol, 1.18% nicotine, <5% tobacco essence.
[5] No data are presented for 'control' studies; findings are reported descriptively in the narrative.

changes compared with 'screening' for any of the measured parameters, either in the asthmatic or the non-asthmatic group. After 'experimental' exposures, significant changes were measured at T0 vs. 'screening' in all parameters in both groups, except for X5 in non-asthmatics: Z5, R5, R10, R20, f_{res}, AX increased in both groups; X20 decreased in both groups; X5 decreased in asthmatics. The changes showed a similar pattern over time, but were of greater magnitude, in asthmatics compared with non-asthmatics.

12. The fraction of exhaled nitric oxide (FeNO) measurements was also made at 'screening', T0, T15, and T30. Values were not significantly different between groups at 'screening' and did not change significantly after 'control' exposures. After 'experimental' exposures, FeNO fell significantly in both non-asthmatics and asthmatics, with a return to 'screening' levels by T15 in non-asthmatics and T30 in asthmatics.

13. Flouris and colleagues reported that E(N)NDS use did not affect lung function or exhaled NO in current CC smokers. Test subjects (n=15, male and female) used E(N)NDS (Giant, Nobacco GP[6], Greece, 11 mg/mL nicotine) for 30 min (3-14 puffs, depending on the individual participant, median = 11 puffs), or smoked 2 usual brand CCs within 30 min, or sham-smoked an unlit CC during 30 min. The order of tests was randomised and there was a 7-day washout period between tests. Lung function tests (FEV_1, FVC, FEV_1/FVC, PEF) performed immediately and 1 h post exposure showed significantly reduced FEV_1/FVC immediately after CC smoking but no significant changes associated with E(N)NDS use. Plasma cotinine increased significantly after both CC or E(N)NDS use (Flouris et al. 2013).

14. Boulay et al. (2017) evaluated the effects of using a non-nicotine, flavour-free E(N)NDS product for 1 h in healthy and asthmatic subjects, all of whom were non-smokers and non-E(N)NDS users. The test E(N)NDS liquid comprised a 70%/30% PG/VG mixture. Experimental (with cartridge/liquid) or placebo (without) test sessions, performed 1 week apart, consisted of inhaling the test product 3x per min for a duration of 1 h. No significant differences in respiratory mechanics and lung function tests or reported symptoms were associated with the E(N)NDS use in either group compared with baseline values.

4.1.1.2 Cough and mucociliary clearance

15. Dicpinigaitis and colleagues found that cough reflex induced by capsaicin in a group of 30 healthy adult non-smokers was suppressed 15 min after using a nicotine-containing E(N)NDS product (Blu, Classic Tobacco flavour[7]; 30 puffs, 30 s puff interval). Re-testing with a non-nicotine-containing E(N)NDS product ('Full Tobacco flavor; Blue Star'[8]) of the 8 subjects showing the highest levels of cough-

[6] Constituents: >60% PG, <10% nicotine, <5% linalool, <5% tobacco essence, <1% methyl vanillin
[7] Constituents: 'distilled water, nicotine, vegetable glycerin, natural flavors, artificial flavors, and citric acid'. Thirty puffs were estimated to deliver 1.5-1.8 mg nicotine.
[8] Described as having a similar vehicle to the nicotine-containing test product, however actual constituents were not reported.

suppressive effect, using the same protocol, showed no effects on cough reflex inhibition. Authors concluded that a single session of E(N)NDS use could cause inhibition of cough reflex and that this effect was caused by the presence of nicotine (Dicpinigaitis et al. 2016).

4.1.1.3 Gene expression in the respiratory tract

16. Staudt et al. (2018) concluded that acute E(N)NDS use by healthy individuals who were never–CC smokers and never–E(N)NDS users affected gene expression in respiratory epithelium and alveolar macrophages, and, in the case of nicotine-containing E(N)NDS, caused elevated levels of plasma endothelial microparticles. A group of 10 participants, who reported never having smoked CC or used E(N)NDS, underwent, at baseline, a detailed medical examination, including chest X-ray, pulmonary function tests and standard blood and urine tests. Bronchoscopy was performed with brushings to sample the small airway epithelium (10^{th}-12^{th} order bronchi), broncho-alveolar lavage (BAL) to obtain alveolar macrophages, and blood samples were evaluated for the presence of endothelial microparticles. One week later, the subjects underwent exposure to aerosol from a 'Blu' E(N)NDS product: 10 puffs, twice, with a 30-min interval. Subjects were randomised to either 'with nicotine' (n=3) or 'without nicotine' (n=7). The study did not include a control (non-E(N)NDS exposure) group, nor were details given of the constituents of the e-liquid[9] or of the concentration of nicotine in the nicotine-containing e-liquid.

17. Immediately after the E(N)NDS exposure, vital signs and oxygen saturation were evaluated and a clinical symptoms questionnaire was completed by the participants. Within the next 2 h, bronchoscopies and BAL were again carried out and findings were compared with those from the baseline investigations performed one week previously. The authors reported that 'there were no consistent changes in vital signs, lung function tests, O_2 saturation, blood carboxyhemaglobin levels or urine nicotine metabolite levels, BAL cell differentials or small airway epithelium cell differentials'. They did, however, find that, compared with baseline measurements, levels of endothelial microparticles were elevated in the set of three subjects exposed to nicotine-containing E(N)NDS aerosol (but not in the non-nicotine group), and that there were alterations in gene expression in small airway epithelium and alveolar macrophages observed in the groups of subjects exposed to E(N)NDS, both with (52 downregulated, 19 upregulated genes) and without (25 downregulated, 40 upregulated genes) nicotine. The authors concluded that 'this study provides *in vivo* human data demonstrating that acute inhalation of EC aerosols dysregulates normal human lung homeostasis in a limited cohort of healthy naïve individuals'.

4.1.2 Cardiovascular effects

18. A number of clinical studies have investigated short-term cardiovascular effects of E(N)NDS exposures, including heart rate, blood pressure, arterial stiffness, biomarkers of oxidative stress, endothelial function, endothelial progenitor cells and

[9] 'Blu' is a brand name for which many different products are available.

microvesicles, autonomic control and heart-rate variability, and cardiac geometry and function. The studies described below are grouped by type of E(N)NDS device tested (which may play a role in the effectiveness of nicotine delivery to the user).

4.1.2.1 First generation devices

19. Eissenberg (2010) examined the effects of E(N)NDS aerosol exposure ('NPRO' or 'Hydro', with 16 mg nicotine cartridge, menthol or regular flavour[10]) on heart rate. Healthy adult male and female CC smokers (n=16), who had a/bstained from tobacco/nicotine exposure for 12 h, took 10 puffs *ad lib* at times 0 and 60 min. Heart rate and plasma nicotine levels were measured at 5, 15, 30, and 45 min after each exposure session. Tests were also carried out with own-brand CC and with sham smoking (unlit CC) in a 4 Latin-square ordered conditions with a 48-h washout period. Authors reported that significant increases in heart rate were only observed at 5 min and 15 min after smoking own-brand CC, although data were not shown. E(N)NDS use produced no or a relatively small increase in plasma nicotine compared with CC.

20. The group of Eissenberg reported a similar study to that reported by Eissenberg (2010) (described in paragraph 19, above) in a group of 32 adult male and female CC smokers. In this 4-arm study, test products were 'NPRO'[11] with 18 mg nicotine test cartridge or 'Hydro'[12] with 16 mg nicotine test cartridge, with flavours matched to the participant's usual brand of CC (regular or menthol). Ten puffs were taken with a 30-s inter-puff interval and heart rate measurements were made at 20-s intervals (duration not described) after exposure. Significant increases in heart rate and plasma nicotine concentration compared with baseline values were only observed after CC use (Vansickel et al. 2010).

21. Cooke et al. (2015) evaluated the effect of E(N)NDS nicotine on arterial blood pressure in young, adult non-smokers (n=20, male and female). Subjects were exposed to 0 (placebo) or 18 mg/mL nicotine from a 'Green Smart Living' or 'Clean Electronic Cigarettes' device[13]: 1 puff every 30 s during 10 min. The study was a randomised, double-blind, 2-arm crossover, with a 1-week washout period. Nicotine exposure was associated with significantly increased systolic blood pressure compared with placebo, and with increased diastolic blood pressure compared with baseline and placebo values.

22. Yan and D'Ruiz (2015) evaluated the effects of 'blu' cigalike E(N)NDS with 5 different e-liquids (varying PG/VG ratios; 1.6-2.4% nicotine; different flavours), compared with a Marlboro Gold King Size CC, on factors including plasma nicotine concentration, heart rate, and blood pressure. Participants (n=23 adults, male and

[10] Other constituents not stated
[11] The report states that 'According to the NJOY website, cartridge ingredients include nicotine, propylene glycol, water, ethanol, glycerol, acetylpyrazene, guaiacol, myosmine, cotinine, and vanillin.'
[12] The report states that 'according to the manufacturer's website, cartridges also come in a variety of flavours and contain nicotine (0-16 mg), propylene glycol, water, and tobacco flavouring.'
[13] Constituents other than nicotine were not described

female) used E(N)NDS (50x 5-s puffs at a 30-s puff interval or *ad lib* for 1 h) or smoked CC (1 CC or *ad lib* for 1 h). Plasma nicotine, heart rate, and blood pressure were all increased after test exposures, although values were generally higher after CC than E(N)NDS use. The authors noted a trend for correlation between plasma nicotine level and increased heart rate.

23. Fogt et al. (2016) assessed the effect of nicotine delivered by E(N)NDS on blood pressure, metabolic rate, metabolic responses and aerobic power in young, normotensive non-smokers (10 men and 10 women). Participants took 20 puffs of E(N)NDS ('Green Smart Living') with a 30-s puff interval. The study was carried out in a 2-arm, double-blind, crossover design, using E(N)NDS devices with cartridges containing either 0 mg nicotine or 18 mg nicotine[14]. At 40 min post exposure, resting diastolic blood pressure was significantly increased and resting systolic blood pressure was significantly reduced after 18 mg nicotine compared with 0 mg nicotine E(N)NDS exposure. There were no significant differences in other test parameters between nicotine and placebo exposures.

24. Moheimani et al. (2017a) carried out an open-label, randomised, crossover study including 33 healthy volunteers who had not smoked CC or used E(N)NDS for ≥ 1 y. Participants used either a 'Greensmoke' cigalike[15] (n=15) or a second-generation 'eGo' device[16] (n=18), both with and without 1.2% nicotine, 1 puff per 30 s during either 10 min (n=6 cigalike) or 30 min (n=27), or a sham control (device without liquid[17]). Measurements (blood pressure, heart rate, electrocardiogram (ECG), blood sampling) were made before and immediately after exposures. Heart rate variability components reflecting vagal, sympathetic activity and sympathovagal balance (measured on ECG) showed a shift towards sympathetic predominance (compared with baseline) after E(N)NDS + nicotine, but not with the non-nicotine device or sham control. Plasma paraoxonase, a marker of oxidative stress, did not increase after any of the exposures. Authors concluded that the acute sympathomimetic effect of E(N)NDS is caused by the inhaled nicotine.

4.1.2.2 Second-generation devices

25. Farsalinos et al. (2014) used ECG examinations to evaluate the acute effects of E(N)NDS use in comparison with CC smoking. Participants were heavy smokers (n=36; 3 female) or E(N)NDS users (ex-smokers who had switched to E(N)NDS for ≥ 1 month) (n=40; 3 female), with no cardiovascular disease (CVD) risk factors. After 4 h abstinence from caffeine, alcohol, CC smoking, and E(N)NDS use, CC smokers smoked 1 CC while E(N)NDS users used an eGo device with 11 mg/mL nicotine e-liquid[18], *ad lib* for 7 min. Measurements were made 5 min later. Baseline characteristics of both groups were similar. CC smoking was associated with

[14] The report notes that 'The 18 mg and 0 mg EC cartridges are marketed to vary only in nicotine content.'
[15] tobacco-flavoured liquid, VG/PG solvents
[16] strawberry flavour, VG/PG solvents
[17] further details not given
[18] Containing PG > 60%, linalool < 5%, nicotine < 10%, tobacco essence < 5%, methyl vanillin < 1%.

significant increase in systolic and diastolic blood pressure, heart rate, and parameters of left ventricular myocardial function, while E(N)NDS use was associated only with increased diastolic blood pressure.

26. Antoniewicz et al. (2016) reported that exposure to E(N)NDS aerosol (10 puffs over 10 min of an e-liquid[19] containing 12 mg/mL nicotine) was associated with significantly increased numbers of endothelial progenitor cells in blood at 1 h post exposure, compared with baseline, in a randomised, 2-arm, crossover study (1-week washout) of 14 young healthy 'seldom CC smokers'. The authors postulated that this could impact on vascular integrity, leading to future atherosclerosis.

4.1.2.3 Tank systems

27. St Helen et al. (2017) reported that e-liquid flavour may influence nicotine absorption from inhaled E(N)NDS aerosol. Exposure to a strawberry-flavoured product led to a mean maximum increase in heart rate of 17.2 beats/min during the 30 min after 15 puffs (uncontrolled puff duration) from a KangerTech 3.7 V, 1000 mAh battery E(N)NDS tank system device (18 mg/mL nicotine, 50/50 PG/VG, strawberry flavour) in experienced E(N)NDS users. In comparison with use of this strawberry-flavoured product, mean maximum heart rate increases were lower on use of a tobacco-flavoured e-liquid (12.3 beats/min) or participant's own brand e-liquid (9.4 beats/min), which the authors suggested to be correlated with different nicotine exposure kinetics related to the flavour characteristics (e.g. e-liquid pH).

28. Spindle et al. (2017) reported that E(N)NDS use (user's own device, all tank systems, ≥ 12 mg/mL nicotine), after 10 puffs with a 30-s puff interval, then *ad lib* use for 90 min, was associated with significantly increased plasma nicotine concentration and heart rate, measured during the period immediately after exposure, in 29 healthy, adult regular E(N)NDS users (mostly male).

4.1.2.4 Mixed

29. St Helen et al. (2016) reported that E(N)NDS use (15 puffs of participant's usual brand[20] – all with nicotine – at a 30-s puff interval) increased heart rate, measured 5 min after exposure, by an average 8.0 beats/min in a group of 13 healthy adult male and female experienced E(N)NDS users.

4.1.2.5 Unspecified

30. Carnevale et al. (2016) reported that single use of E(N)NDS or CC affected markers of oxidative stress and flow-mediated dilation (used to assess endothelial function). Healthy subjects (20 smokers and 20 non-smokers, age- and sex-matched) first smoked 1 CC, then one week later took 9 puffs from a 'leading brand

[19] 49.4% PG, 44.4% glycerin, 5% ethanol, 1.2% nicotine, with no flavouring; eGo device at 3.7 V.
[20] 2x first-generation, 8x second-generation, and 3x rebuildable atomizer devices, all e-liquids contained PG and VG but contents were not otherwise specified.

e-cigarette'[21] with a 16 mg nicotine cartridge. Measurements were made before and 30 min after exposures. The study was single-blind (laboratory analyses of blood samples and flow-mediated dilation measurements). Smokers showed higher oxidative stress levels measured at baseline. Significant increases in markers of oxidative stress (soluble NOX2-derived peptide (sNOX2-dp), 8-iso-prostaglandin F2a (8-iso-PGF2a) and significant decreases in vitamin E, NO bioavailability, and flow-mediated dilation values were measured in both non-smokers and smokers after E(N)NDS or CC use. Statistical analysis determined that the effect of E(N)NDS was less than that of CC for sNOX2-dp, 8-isoPGF2a, and NO bioavailability.

4.2 Animal data

31. One study was identified that reported effects of acute exposure to E(N)NDS aerosol on respiratory epithelium in mice.

32. Western blot analysis of nasal epithelial tissue harvested from C58/B16J mice (n=6/group (sex not stated)) exposed for 3 h in a chamber to a 50:50 PG/VG 'vapour' showed significantly increased levels of Mucin 5AC, Oligomeric Mucus/Gel-Forming (MUC5AC) and stromal interaction molecule-1 (STIM1) proteins in comparison with room-air–exposed controls. The report stated that 'Vapor was generated using 100 W power. Mice were exposed to puffs of 3 s duration with an interval of 10 min between puffs.' Further details of the exposure protocol were not given. Authors concluded that these findings, taken together with results of analyses of bronchial brush biopsies and lavage samples from human E(N)NDS users (see paragraph 39), indicated that chronic E(N)NDS aerosol exposure exerts biological effects on the lung, which may in part be mediated by the PG/VG base (Ghosh et al. 2018).

5 Repeat-dose toxicity

5.1 Humans

33. Literature relating to the health effects of E(N)NDS aerosols in humans was mostly found to relate to effects on the respiratory system, including clinical case reports, clinical studies of E(N)NDS users in comparison with CC smokers and/or non-smokers, and some cross-sectional epidemiological studies. In addition, a small number of reports were identified relating to potential effects of repeated exposure to E(N)NDS aerosol exposure on oral/periodontal health and on the cardiovascular system.

5.1.1 Clinical case reports and case series

34. Hua and Talbot (2016) summarised a series of case reports concerning potential health effects of E(N)NDS exposure in 26 individuals (27 publications), identified by searching the literature published between April 2012 and January 2016. Of these, 12 described nicotine poisoning by accidental or deliberate ingestion of E(N)NDS liquids, 2 related to mechanical injury from battery explosion, and 13

[21] Further details not given.

cases related to inhalation of E(N)NDS aerosols. These latter 13 cases included 2 reports associating E(N)NDS use with potential amelioration of clinical symptoms (remission of ulcerative colitis and of chronic idiopathic neutrophilia) and 11 cases in which E(N)NDS use was associated with negative health outcomes: 6 respiratory, 2 gastrointestinal, 2 cardiovascular, 1 neurological. Four cases described worsening of a pre-existing condition.

35. Additional reports identified from a literature search include one case suggesting beneficial effects of E(N)NDS use on tonsillitis (Miler and Hajek 2017), one case reporting a potential negative association of E(N)NDS use with post-surgical healing (Fracol et al. 2017), and 3 further cases of respiratory effects associated with E(N)NDS use (Madsen et al. 2016, Flower et al. 2017, Khan et al. 2018). A summary of case reports relating to inhalation of E(N)NDS products is given in Table 1.

Table 1. Case reports of health effects associated with E(N)NDS use.

	Patient description	**Presentation / diagnosis**	**E(N)NDS use, if described**	**Follow-up**
Respiratory				
McCauley et al. (2012)	Adult female with schizoaffective disorder	Lipoid pneumonia with symptoms for 7 months	7-month history of E(N)NDS use	Symptoms improved on medical treatment and cessation of E(N)NDS use
Hureux et al. (2013)	Adult male with pulmonary lung adenocarcinoma and isolated brain metastasis; CC smoker attempting to quit	Bronchiolitis starting within 3-7 days of E(N)NDS use	125-150 puffs/day E(N)NDS with nicotine	Symptoms resolved within 7 days of stopping E(N)NDS use
Thota et al. (2013)	Young healthy adult male	Acute eosinophilic pneumonia starting within 3-7 days of E(N)NDS use	-	Resolved on medical treatment
Atkins and Drescher (2015)	Adult male with history of medical admittance for respiratory concerns	Suspected acute hypersensitivity pneumonitis	-	Improved after medical treatment and ceasing E(N)NDS use

	Patient description	**Presentation / diagnosis**	**E(N)NDS use, if described**	**Follow-up**
Modi et al. (2015)	Adult female	Lipoid pneumonia	-	Improved after medical treatment and cessation of E(N)NDS use
Moore et al. (2015)	Adult male with history of hypertension; CC smoker	Pneumonia with bilateral pleural effusions starting within 3-7 days of E(N)NDS use	'hundreds of puffs per day' for 3 days	Resolved after medical treatment including antibiotics
Madsen et al. (2016)	Adult female; ex-CC smoker	Abdominal pain and fever, multiple pulmonary nodules and liver lesions indicative of metastasis on imaging. Multinucleated giant cells on lung biopsy revealed suggested foreign body reaction to lipophilic material.	10 mL/week E(N)NDS use for previous 20 months after quitting CC smoking	Disappearance of lung nodules and regression of liver lesions on discontinuation of E(N)NDS use.
Flower et al. (2017)	Adult male; CC smoker; mixed germ cell tumour	Respiratory bronchiolitis interstitial lung disease diagnosed by CT tomography and open lung biopsy	E(N)NDS use 10-15 times per day plus continued CC smoking during 3 months	Radiographic improvement on cessation of E(N)NDS use and reduction of CC smoking
Itoh et al. (2018)	Healthy adult male; ex-CC smoker	Acute alveolitis: intra-alveolar fibrosis with exudate containing lipid-laden macrophages, eosinophils, and neutrophils. Two-month duration. Diagnosed as E(N)NDS-induced injury.	E(N)NDS use at equivalent of 20 CC per day (e-pen) after quitting CC smoking 3 months previously	Symptoms resolved after corticosteroid therapy and discontinuation of E(N)NDS exposure

	Patient description	**Presentation / diagnosis**	**E(N)NDS use, if described**	**Follow-up**
Khan et al. (2018)	Adult female; CC smoker attempting to quit	Organising pneumonia and acute respiratory failure preceded by 1 month of dyspnoea and chest pain	Use of E(N)NDS during the previous month	Recovery after treatment with corticosteroids and cessation of E(N)NDS use
Gastrointestinal				
Camus et al. (2014)	Adult female with ulcerative colitis; CC smoker	Relapse of ulcerative colitis with E(N)NDS use	10 mL E(N)NDS liquid vaped per 5 days, with nicotine	Improvement after stopping E(N)NDS and recommencing CC smoking
Gillen and Saltzman (2014)	Infant	Necrotizing enterocolitis	Exposure to E(N)NDS *in utero*; mother used E(N)NDS approximately 30-50 times per day during pregnancy	Recovery after surgery
Cardiovascular				
Monroy et al. (2013)	Elderly adult female with hyperlipidaemia, osteoarthritis, allergic rhinitis, previous history of breast adenocarcinoma and hip fracture; CC smoker attempting to quit	Episodes of atrial fibrillation during E(N)NDS use		Episodes of atrial fibrillation stopped after medical treatment and discontinuation of E(N)NDS use
Kivrak et al. (2014)	Healthy young adult male; CC smoker attempting to quit for 1 month	Acute myocardial infarction with symptoms beginning during E(N)NDS use	Tobacco-flavoured E(N)NDS product with nicotine	Recovery after medical treatment
Neurological				
Vannier et al. (2015)	Healthy adult male; CC smoker	Reversible cerebral vasoconstriction syndrome; headaches and seizures, onset 2 days after starting E(N)NDS use	E(N)NDS with nicotine + CC smoking	Resolved with medical treatment and cessation of E(N)NDS use (continued CC smoking)

	Patient description	**Presentation / diagnosis**	**E(N)NDS use, if described**	**Follow-up**
Surgical wound healing				
Fracol et al. (2017)	Adult female; ex–CC smoker undergoing bilateral mastectomy for non-metastatic ductal carcinoma *in situ*	Patient developed extensive post-operative bilateral skin flap necrosis. Authors noted that nicotine is known to increase risk of skin flap necrosis and surgical site infection and cautioned that further knowledge should be gained about the potential effects of E(N)NDS use in individuals undergoing surgery.	Switched from CC smoking to E(N)NDS use approximately 3 months prior to surgery (1.5-pack per day equivalent). Authors noted that CC smoking has significant effects on post-operative healing, that E(N)NDS use may have similar effects, and that nicotine may, theoretically, be involved	
Improvement of disease status reported with E(N)NDS use				
Lee et al. (2013)	Adult male with history of ulcerative colitis which began 4 weeks after CC smoking cessation	Clinical remission of ulcerative colitis	Mean 105 puffs E(N)NDS aerosol per day (nicotine concentration was not noted)	
Farsalinos et al. (2013)	Adult male with chronic idiopathic neutrophilia, hyperlipidaemia (on treatment); CC smoker	Leucocyte and C-reactive protein normalised on starting E(N)NDS use and quitting CC smoking	E(N)NDS with nicotine	
Miler and Hajek (2017)	Young adult female never smoker with frequent episodes of tonsillitis and recurrent tonsilloliths	Resolution of chronic tonsillitis and improvement in tonsilloliths after approximately 3 months of E(N)NDS use	Several months of E(N)NDS use, 8-20 mL per day of 0-3 mg/mL nicotine, varied flavours	8 months after starting vaping the improvement in symptoms was still evident

For all reports except Madsen et al. (2016), Flower et al. (2017), Fracol et al. (2017), Miler and Hajek (2017), Itoh et al. (2018), and Khan et al. (2018), the information was obtained from the review of Hua and Talbot (2016).

5.1.2 Effects on the respiratory system

36. Exposure to E(N)NDS aerosol has the potential, in theory, to damage the respiratory system or exacerbate pre-existing lung disease. A variety of possible mechanisms whereby this might occur were discussed in the recent review of the 'Public Health Consequences of E-cigarettes' by the U.S. National Academy of Sciences (NAS 2018). These include a potential impact of nicotine on host-defence mechanisms in the lungs and immune response, impaired cough, and impaired mucociliary clearance (MCC) (via impairment of cystic fibrosis transmembrane conductance regulator (CFTR)). Potential effects of other constituents (ultrafine particles, oxidants, flavourings and other chemicals) were considered to include damage to the airways and lung parenchyma, to cause inflammation, bronchoconstriction, leading to airway remodelling and/or asthma, and ultimately chronic obstructive pulmonary disease (COPD) and end-stage lung disease.

37. There is currently a limited data-set regarding effects on the respiratory system of E(N)NDS exposure in humans, and interpretation of the data is complicated by such factors as the wide variation in test product constituents, test procedures, subject characteristics, and delivery systems used. Many studies to date have focussed on the potential reduction in harm associated with E(N)NDS use in comparison with CC smoking and/or on effects of E(N)NDS exposure on short-term endpoints, such as respiratory function parameters and self-reported respiratory symptoms. Questions that may need to be addressed include effects in other groups, such as non-/never smokers, individuals with pre-existing lung conditions such as COPD or asthma, or different age groups, and potential effects of combined E(N)NDS/CC use (*discussed by* (NAS (2018)).

5.1.2.1 Evaluation of respiratory epithelial samples

38. Reidel et al. (2018) investigated the possible effects of E(N)NDS use or CC smoking on protein levels in sputum. Induced sputum samples were collected from CC smokers[22] (n=14), E(N)NDS users[23] (n=15, of whom 12 were ex-CC smokers and 5 reported current, occasional CC use), and never smokers (n=15). Compared with never smokers, 81 and 41 proteins showed altered abundance (either increased or decreased) in mucus of CC smokers and E(N)NDS users, respectively, with some overlapping patterns of change and also some distinct patterns of changes observed between CC smokers and E(N)NDS users. In comparison with never smokers, changes that were highlighted in the report were higher levels of secreted proteins related to the innate defence functions of leucocytes in E(N)NDS users only, and

[22] Average 11 CC/day
[23] Exclusive or predominant E(N)NDS use for at least 6 months; average 280 puffs/day

increased levels in both E(N)NDS users and CC smokers of secondary neutrophil granule proteins. Sputum from E(N)NDS users also showed increased levels of neutrophil extracellular trap (NET) proteins. Total mucin concentrations were significantly increased in sputum from CC smokers but not E(N)NDS users compared with never smokers. Authors concluded that 'e-cigarette use alters the profile of innate defence proteins in airway secretions, inducing similar and unique changes relative to cigarette smoking'.

39. Ghosh and Bradley Drummond (2017) compared bronchial brush biopsies and lavage samples taken from non-smokers (n=18), CC smokers (n=13) and E(N)NDS users (n=10). Subjects were young[24], healthy, adult males and females. CC smokers smoked an average of 10.1 ± 4.1 CC per day (average 9.5 ± 6.2 pack years), with little or no E(N)NDS use. E(N)NDS users had switched to E(N)NDS for at least 6 months, took a mean 44.1 ± 82.2 puffs/day[25], and included 2 subjects who also smoked CC (< 5 per week). In non-smokers the airway mucosa was healthy in appearance, compared with 'more erythematous and irritable airway mucosa' in CC smokers and E(N)NDS users. Proteomic analysis of brush biopsies (n=8 non-smokers, n=9 CC smokers, n=9 E(N)NDS users) indicated approximately 300 proteins that were differentially expressed in CC smokers and E(N)NDS users compared with non-smokers, with 78 proteins common to both groups (including upregulation of MUC5AC and Vesicle Associated Membrane Protein 8 (VAMP8)) and expression of 113 proteins altered only in E(N)NDS users (including upregulation of MUC4). Fourteen pathways, which included proteins involved in organelle membranes, early endosomes/trafficking, macromolecular complexes, and mitochondria, were altered only in E(N)NDS users.

5.1.2.2 Cough and mucociliary clearance

40. Kumral et al. (2016) concluded that using E(N)NDS to quit smoking has negative effects on sino-nasal symptoms and MCC. In this single-blind study, a total of 98 adult male and female smokers (1 pack per day for at least 5 y) admitted to a smoking cessation clinic in Turkey were randomised to quit smoking using E(N)NDS (group 1, n=42 included in final analysis[26]) or cognitive behavioural treatment (group 2, n=30 in final analysis). Choice of E(N)NDS device and liquid was made individually, with nicotine concentrations in the range 11-12 mg/mL. Participants completed a sino-nasal outcome test (SNOT-22) (sino-nasal symptoms, evaluated by questionnaire; a higher score indicates a higher level of symptoms of nasal disorder) and saccharin transit test (to indicate nasal MCC) at baseline and 3 months after quitting CC. There were no statistical differences in SNOT-22 scores and MCC between groups at baseline. At 3 months, SNOT-22 scores had significantly decreased in both groups vs baseline, and were significantly lower in group 2 than group 1. At 3 months, MCC in group 2 was significantly lower than at baseline and

[24] CC smokers were significantly older than the other two groups.
[25] Mean e-liquid consumption in E(N)NDS users was 11.4 ± 17.0 mL per day
[26] Patients who managed to quit CC smoking during the study period.

was significantly lower than in group 1. There was no significant change in MCC during the 3-month period in group 1.

5.1.2.3 Evaluation of respiratory function in CC smokers switching to E(N)NDS

41. A number of studies have assessed respiratory function in CC smokers, with or without existing respiratory disease, who have taken up E(N)NDS use (with or without continuation of CC use).

42. A group of researchers has reported a series of such studies carried out in Italy (Campagna et al. 2016, Cibella et al. 2016, Polosa et al. 2014a, Polosa et al. 2014b, Polosa et al. 2016b, Polosa et al. 2016c). The recent review by NAS concluded that these studies suggest that smokers with pre-existing lung conditions such as asthma and COPD may experience some benefits from switching to E(N)NDS. These benefits have been reported as increased FEV_1, improved performance in methacholine challenge test for asthma, and decreased exacerbations of COPD. However, NAS noted that these studies had limitations including retrospective design and small sample size (NAS 2018).

43. A separate study carried out in the UK found no difference in lung function in a group of around 400 CC smokers with no pre-existing respiratory conditions who were randomised to either switch to E(N)NDS ('Puritane', comprising approximately 70% PG, 30% VG, 2% nicotine, tobacco or menthol flavour) or continue smoking CC for 12 weeks, although it was noted that some participants in the E(N)NDS group actually dual-used E(N)NDS and CC (Cravo et al. 2016).

44. A follow-on from the study of Cravo et al. (2016) described the evaluation of tolerance of the Puritane E(N)NDS product in the same study group over a 2-year follow-up period. Outcomes assessed were adverse events, vital signs, ECG, lung function tests, exposure to nicotine and other smoke constituents, and parameters of desire to smoke. The authors noted that no serious adverse events were reported and that the aerosol was well tolerated and not associated with any clinically relevant health concerns after use for up to 24 months. Spirometric tests indicated small but statistically significant decreases in all four of the lung function test parameters in comparison with baseline values, but the authors considered that these changes were not clinically significant, postulating that they may be due to factors such as subject aging during the 2 y of the study or over-motivation to perform well at baseline testing (Walele et al. 2018).

5.1.2.4 Epidemiological studies of asthma and respiratory symptoms in adolescents

45. Some cross-sectional studies, based on self-reported information from questionnaire surveys, have observed associations between E(N)NDS use and asthma and/or other respiratory symptoms in adolescents.

46. Analysis of data from the 2012 Florida Youth Tobacco Survey indicated that asthma was more common in adolescents in Florida who were current or previous

E(N)NDS users compared with never users (Choi and Bernat 2016). Questionnaire data from high school students (n=36,085) included information on ever diagnosis of asthma and current asthma status, asthma attack during the previous 12 months, and E(N)NDS and/or CC use, ever or within the last 30 days (current). The proportion of overall study population who were ever E(N)NDS users (n=3185) was 8.2% and that of current E(N)NDS users (n=1320) was 3.3%. E(N)NDS use was higher in participants reporting asthma (10.4% had ever used E(N)NDS and 5.3% were current E(N)NDS users) compared with those 'never diagnosed with asthma' (7.2% had ever used E(N)NDS and 2.5% were current E(N)NDS users)[27]. Authors also reported that 'Past 30-day e-cigarette use was associated with having an asthma attack in the past 12 months among participants with asthma (n=5865; p<0.01).' E(N)NDS use vs. no E(N)NDS use within the last 30 days was associated with reporting an asthma attack within the previous 12 months (adjusted[28] OR=1.78; 95% confidence interval (CI), 1.20-2.64). However, it is difficult to evaluate the various associations reported by the authors as data are not clearly presented in the publication.

47. Cho and Paik (2016) found an association between asthma and E(N)NDS use in adolescents in South Korea. Data were obtained from the Tenth Korean Youth Risk Behavior Web-based Survey (KYRBWS) in 2014. High school students in 10th-12th grade (n=35,904; mean age 16.4 years) completed a questionnaire including whether they had been diagnosed with asthma by a doctor within the last 12 months, number of days absence from school with asthma during the past 12 months (as a measure of asthma severity), ever use and current use (within 30 days) of E(N)NDS and of CC. Overall, 674 (1.9%) of the study population had been diagnosed with asthma within the last 12 months. Of these, 98 were current E(N)NDS users (3.9% of n=2513 current E(N)NDS users), 46 were previous E(N)NDS users (2.2% of n=2078 previous E(N)NDS users), and 530 had never used E(N)NDS (1.7% of n=31,313 never E(N)NDS users). Regarding CC smoking, 150 and 524 asthmatics were described as CC smokers and non-smokers, respectively[29].

48. The unadjusted OR for asthma in current vs. never E(N)NDS users was 2.36 (95% CI, 1.89-2.94), adjusted for gender the OR was 2.09 (95% CI, 1.67-2.62), and adjustment for CC smoking produced an OR of 1.73 (95% CI, 1.28-2.34). After stratification for smoking (never, former, current), within the never smokers group the unadjusted OR for asthma in current vs. never E(N)NDS users was 3.41 (95% CI, 1.79-6.49) and the adjusted[30] OR was 2.74 (95% CI, 1.30-5.78).

49. Current E(N)NDS use was also associated with increased school absence due to asthma. Using multi-nominal logistic regression analysis of the frequency of

[27] It is unclear exactly what these percentages represent as the data relating to numbers of study particapants to which they refer are not given in the publication.
[28] Adjusted for age, race/ethnicity, gender, metropolitan status, days smoked cigarettes in the past 30 days, positive social norm towards smoking, and exposure to second-hand smoke.
[29] It is not clear whether 'former smokers' are included in the 'smokers' or 'non-smokers' group.
[30] Adjusted for gender, city size, multi-cultural family status, overweight, second-hand smoking, atopic dermatitis history, allergic rhinitis history.

students' absence from school for asthma[31], within the never CC smokers group, ORs for > 4 days absence from school (taken as an indicator of severe asthma) were 18.59 (95% CI, 7.23-47.82) unadjusted and 15.42 (95% CI, 5.11-46.57) adjusted for current E(N)NDS users. For former E(N)NDS users, the ORs were 2.03 (95% CI, 0.28-14.81) unadjusted and 1.63 (95% CI, 0.22-12.15) adjusted.

50. An evaluation of previous KYRBWS data (2011, 2012, 2013) by Kim et al. (2017) also indicated a positive relation of E(N)NDS use with asthma (adjusted[32] OR=1.12, 95% CI, 1.01-1.27), although the authors noted that in this analysis, effects of past smoking history could not be excluded.

51. Wang et al. (2016) also found that respiratory symptoms were more prevalent in association with E(N)NDS use in Chinese adolescents in Hong Kong. Based on completed questionnaires from the Global Youth Tobacco Survey, data were available from 45,128 students (mean age, 14.6 years), of whom 1.1% had used E(N)NDS within the last 30 days. E(N)NDS use was associated with a higher prevalence of respiratory symptoms (cough or phlegm for 3 consecutive months) overall (adjusted[33] OR=1.28, 95% CI 1.06-1.56), and by breakdown in non-smoker (adjusted odds ratio (AOR)=2.06, 95% CI 1.24-3.42), ever smoker (AOR=1.39, 95% CI 1.14-1.70), and ex-smoker (AOR=1.40, 95% CI 1.02-1.91) groups. The association was not statistically significant in current smokers (AOR=1.15, 95% CI 0.81-1.62).

52. McConnell et al. (2017) reported that E(N)NDS use was associated with increased rates of chronic bronchitic symptoms in Californian adolescents. In 2014, participants in the Southern California Children's Health Study (n=2086) in 11th and 12th grade (mean age, 17.3 years) completed a questionnaire on E(N)NDS use, tobacco product use, and symptoms of chronic bronchitis (chronic cough, phlegm, bronchitis) and wheeze during the previous 12 months. A total of 502 participants had ever used E(N)NDS, of whom 301 were previous users and 201 were current users (24%, 14.4%, and 9.6%, respectively). Compared with never use, bronchitic symptoms were correlated with past (OR=1.85, 95% CI 1.37-2.49) and current (OR=2.02, 95% CI 1.42-2.88) E(N)NDS use, with risk higher with higher frequency of current use (OR=1.66, 95% CI 1.02-2.68 for 1-2 days and OR=2.52, 95% CI 1.56-4.08 for ≥ 3 days use in the previous 30 days). Analysis restricted to never CC smokers indicated an association of bronchitic symptoms with past E(N)NDS use (OR=1.70, 95% CI 1.11-2.59), while results were not statistically significant in current E(N)NDS users/never CC smokers (OR=1.52, 95% CI 0.89-2.61). No association was observed between E(N)NDS use and wheeze.

53. Schweitzer et al. (2017) found that E(N)NDS use was associated with asthma in a cross-sectional study of adolescents in Hawaii. Data on E(N)NDS, CC, and

[31] The reference category was 'no asthma symptom' group.
[32] Adjusted for age, physical exercise, sex, obesity, region of residence, economic level, educational level of father, educational level of mother, active and passive CC smoking.
[33] Adjusted for sex, age, perceived family affluence, second-hand smoke exposure, and school clustering effects.

marijuana use were collected from the 2015 Hawaii Youth Risk Behavior Survey (HYRBS). A total of 6089 adolescents (50% female, mean age 15.8 y) were included, of whom 22% reported current asthma[34]. For E(N)NDS, 45% were ever users, and 25% of these had used within the last 30 days (current). CC and marijuana were ever used by 25% and 33%, respectively, of whom 10% and 19%, respectively, had used within the last 30 days. In the multivariate analysis, with co-variables including smoking, marijuana use, and other demographic factors, the adjusted OR for the contrast 'current asthma vs. never asthma' in current E(N)NDS users (with 'persons who do not report current use' as the referent group) was 1.48 (95% CI, 1.24-1.78). The adjusted OR for the contrast 'current asthma vs. never asthma' in subjects who had ever used E(N)NDS (never E(N)NDS users were the referent group) was 1.22 (95% CI, 1.01-1.47). Current CC use was not associated with a significantly altered adjusted OR for the contrast 'current asthma vs. never asthma' (referent group, 'persons who do not report current use') (OR=1.23, 95% CI 0.92-1.64), while ever CC use was associated with a significantly increased adjusted OR for 'current asthma vs. never asthma' (never CC use was the referent group) (OR=1.27; 95% CI 1.05-1.54). There were no statistically significant associations between marijuana use and asthma.

54. NAS (2018) concluded that 'There is moderate evidence for increased cough and wheeze in adolescents who use e-cigarettes and an association with e-cigarette use and an increase in asthma exacerbations.'

5.1.2.5 Vulnerable groups

55. The review by NAS (2018) identified the following sub-populations as being potentially particularly sensitive to adverse effects on the respiratory system from E(N)NDS exposure:

- Individuals with COPD (if E(N)NDS use causes additional airway inflammation of already damaged lungs, or promotes continued CC smoking)
- Individuals with asthma, notably adolescents (noting that smoking and air pollution are associated with accelerated decrease in lung function; a point of interest is whether E(N)NDS use can cause neutrophilic inflammation; it was noted that cross-sectional studies have indicated that adolescents using E(N)NDS may be more likely to have increased respiratory symptoms and exacerbations compared with non-users)
- Children and adolescents with cystic fibrosis (as a potential effect of nicotine exposure)
- Preterm infants whose mothers used E(N)NDS during pregnancy (as a potential effect of nicotine exposure *in utero*, given that maternal CC smoking

[34] Authors noted that these prevalences are high (national average in USA for 2015 being 7.8%), but are close to rates reported for Hong Kong and Florida in the studies of Choi and Bernat (2016) and Wang et al. (2016), respectively.

during pregnancy has been associated with the development of bronchopulmonary dysplasia and subsequent respiratory morbidity in pre-term infants).

5.1.3 Oral and periodontal health

56. A few studies have evaluated periodontal health in E(N)NDS users, either in the context of comparison with CC smokers, or evaluations of CC smokers switching to E(N)NDS use. One cross-sectional epidemiological study evaluated some oral health parameters in adolescent E(N)NDS users.

57. Reuther et al. (2016) reported that E(N)NDS use during 5 min was associated with a subsequent small increase in blood flow in the buccal mucosa associated with use of a nicotine-containing E(N)NDS but not with a non-nicotine product. Test subjects were a group of 10 non-CC smokers.

58. Wadia et al. (2016) reported a significant increase in gingival inflammation in CC smokers after switching to E(N)NDS use. Participants (n=18), staff members at a London dental hospital, abstained from CC smoking[35] and instead used blu PRO with tobacco-flavoured e-liquid[36] (18 mg/mL nicotine) for 2 weeks. Bleeding of gums on probing was significantly increased at 2 weeks compared with baseline.

59. Tatullo et al. (2016) reported that oral health parameters (plaque index, bleeding index, papillary bleeding index) improved over time in a pilot study including 110 smokers who reported switching to E(N)NDS use from CC smoking approximately 3-5 months before the beginning of the study. Clinical examinations were performed at baseline, 60 days, and 120 days. In a self-assessment questionnaire, the majority of subjects reported improvements in smell and taste perception and reduced frequency of respiratory diseases, and a feeling of better general health.

60. Javed et al. (2017) reported that some periodontal indices (plaque index, probing depth) in men in Saudi Arabia were poorer in CC smokers (n=33) than E(N)NDS users with no history of CC smoking (n=31) or never smokers (n=30). CC smokers had a mean use time of 5.4 y, whereas mean duration of E(N)NDS use was only 2.2 y.

61. A cross-sectional epidemiological study in Korea evaluated oral health in relation to E(N)NDS use in adolescents (Cho 2017). Data were taken from the 12th Korean Youth Risk Behavior Web-based Survey (KYRBWS), including 65,528 students with a mean age of 15 y. Students were asked to report whether, within the previous 12 months, they had experienced: gingival pain and/or bleeding (18.5%); tongue and/or inside cheek pain (11.0%); a cracked or broken tooth (11.4%). They were questioned on E(N)NDS use and categorised as never users, former users

[35] Four participants did not fully abstain, but smoked < 5 CC during the study period.
[36] Other constituents not described

(5.9%)[37], 1-29 days past month users[38] (1.9%), or daily users (0.5%). Adjusted[39] ORs indicated association of E(N)NDS use with increased incidence of cracked or broken tooth in former (OR=1.16, 95% CI 1.04-1.30, p<0.05), 1-29 days past month (OR=1.26, 95% CI 1.06-1.51, p<0.05), and daily (OR=1.65, 95% CI 1.65-2.27, p<0.01) E(N)NDS users, and with tongue and/or cheek pain in daily users (OR=1.54, 95% CI 1.05-2.26, p<0.05) compared with never users. There were no significant differences for reported gingival pain and/or bleeding.

62. Further analysis indicated different effects of nicotine-containing and non-nicotine-containing E(N)NDS. As compared with subjects who had never used E(N)NDS, use of nicotine-free E(N)NDS during the past 30 days was associated with more tongue and/or inside-cheek pain (adjusted OR=1.56, 95% CI 1.07-2.28, p<0.05), while use of nicotine-containing E(N)NDS was associated with increased reporting of cracked or broken tooth (adjusted OR=1.16, 95% CI 1.04-1.30, p<0.05 for former users; adjusted OR=1.37, 95% CI 1.15-1.63, p<0.01 for use within the past 30 days). The authors considered that these findings would be consistent with findings of DNA strand breaks and cell death caused by E(N)NDS aerosol components in *in vitro* studies (tongue and cheek pain) and known effects of nicotine on tooth structure (cracked or broken tooth).

5.1.4 Cardiovascular system

63. Some of the constituents in E(N)NDS aerosols have the theoretical potential to be associated with the development of CVD, including ultrafine particles, metal particulates, and nicotine. However, there is currently a paucity of epidemiological data to address this aspect, and NAS highlighted the requirement for studies assessing clinical (coronary heart disease, stroke, atherosclerotic peripheral artery disease) and sub-clinical atherosclerosis (carotid intima media thickness and coronary artery calcification) endpoints in relation to E(N)NDS exposures as a major research need (NAS 2018).

64. Although long-term epidemiological data are not available, three longer-term clinical studies were identified.

65. Two of these studies noted an association between CC smoking reduction and reduced blood pressure in hypertensive CC smokers attempting to quit CC smoking by switching to E(N)NDS (Polosa et al. 2016a, Farsalinos et al. 2016).

66. Moheimani et al. (2017b) performed a cross-sectional study including 23 healthy E(N)NDS users (non-CC smokers) and 19 controls (non-CC smokers, non-E(N)NDS users), males and females aged 21-45 y, in California. Heart rate variability and plasma indicators of oxidative stress were measured. A shift towards

[37] Use, but not within the past 30 days.
[38] Use within the past 30 days less frequent than once per day.
[39] All OR values reported here were adjusted for age, gender, school grade, economic status, city size, carbonated drink, overweight status, stress, alcohol, vigorous sports activity, CC smoking, attempt to quit smoking, second hand smoking at home.

sympathetic predominance in cardiac autonomic balance was observed in E(N)NDS users vs. non-users. One of three serum markers of oxidative stress (low density lipoprotein oxidisability) was significantly higher in E(N)NDS users vs. non-users.

5.2 Animal studies

67. Animal studies have indicated that E(N)NDS aerosol exposures have effects on the respiratory system, host-defence against respiratory pathogens, and lung development. However, reports are often limited by lack of standardisation of studies and there is not always adequate reporting of the composition of the products tested and test parameters employed. Some studies have used whole-body exposure, which can lead to exposure by routes other than inhalation (e.g. ingestion via grooming), while others that have used nose restraint may be limited by the effects of stress that this causes to the animals. These aspects are discussed in the review by Chun et al. (2017)

68. Exposure of mice for 3 days to E(N)NDS aerosol was associated with markers of lung inflammatory response and increased oxidative stress. C57BL/6J mice (sex and numbers not reported) were exposed 5 h/day for 3 days to Blu e-cig, Classic tobacco flavour E(N)NDS aerosol with 16 mg nicotine[40], with a reported exposure concentration of approximately 200 mg/m^3 total particulate mass (TPM). Analysis of bronchoalveolar lavage fluid (BALF) showed significantly increased levels of some pro-inflammatory cytokines (monochemoattractant protein (MCP-1), interleukin (IL)-6, IL-1α, IL-13) while lung glutathione levels were decreased, in comparison with air-exposed controls. Macrophage and total cell numbers in BALF were increased over controls, but the difference was not statistically significant (Lerner et al. 2015).

69. Husari et al. (2016) exposed groups of 11 male C57BL/6J mice to E(N)NDS aerosol[41], CC smoke, or laboratory air for 3 days (2 x 3-h sessions per day). The E(N)NDS aerosol was produced from pre-filled 'V4L CoolCart' cartomizer cartridges, labelled as 80/20 PG/VG, 18 mg/mL nicotine, strawberry flavour. TPM in the E(N)NDS aerosol to which the mice were exposed was 1.64 g/m^3, which the authors estimated to be equivalent to approximately 1000 puffs/day from the device used[42]. In comparison with the control group, both E(N)NDS aerosol- and CC smoke-exposed mice showed increased wet/dry lung weight ratio (an indicator of acute lung injury) and increased mRNA expression of the inflammatory mediator, IL-6[43]. Cigarette smoke-exposed mice showed increased albumin leak into BALF, tumour necrosis factor (TNF)-α and IL-1β expression and signs of oxidative stress and apoptotic cell death in the lungs, but these parameters were not different from controls in E(N)NDS aerosol-exposed mice. Histopathological analysis showed inflammatory cell infiltration and alveolar wall changes in CC smoke-exposed mice,

[40] Other constituents not stated
[41] Exposure was described as 'nose-only'.
[42] TPM produced from the aerosol was measured as 74.5 mg/15 puffs.
[43] It is not fully clear from the report whether it is IL-6 or IL-1β expression that is upregulated in lungs of E(N)NDS aerosol-exposed mice, due to inconsistency in reporting between the different parts of the text and in the results presented in Figure 1.

while alveolar structure was described as 'normal' in E(N)NDS aerosol-exposed mice, with a limited focus of infiltration of inflammatory cells.

70. Sussan et al. (2015) reported that exposure to E(N)NDS aerosol impaired immune response and enhanced susceptibility to bacterial and viral infection in mice. Male C57BL/6 mice were whole-body exposed to NJOY menthol bold or traditional bold (1.8% nicotine) E(N)NDS aerosol[44] for 1.5 h, twice per day for 2 weeks. BAL samples showed macrophage infiltration and oxidative stress in the lungs, but no increase in cytokine levels. Intra-nasal infection with *S. pneumoniae* resulted in increased pulmonary bacterial burden in E(N)NDS-exposed compared with. air-exposed mice, although with equivalent neutrophil levels. *Ex vivo* investigations indicated that this was related to impaired phagocytic capability of alveolar macrophages in the E(N)NDS-exposed mice. This effect was noted with both flavours of E(N)NDS product. Experimental infection with H1N1 influenza virus indicated that E(N)NDS-exposed mice responded with increased weight loss and mortality compared with air-exposed controls, associated with increased neutrophilic airway inflammation but decreased cytokine levels. In discussing their findings, the authors noted that although these effects could be partially mediated by nicotine, it is also possible that lipid effects on macrophages (from the E(N)NDS carrier liquid) could impair bacterial phagocytosis.

71. Laube et al. (2017) reported that exposure to nicotine-containing, PG-based E(N)NDS aerosol via an E(N)NDS device (Joyetech 510-T) reduced MCC in mice in comparison with aerosol without nicotine. C57BL/6 mice were whole-body exposed to aerosol of either PG alone or PG/2.4% nicotine, 20 min/day for 1 or 3 weeks. Exposure was achieved from repeat cycles of a 6-s puff (600 µL E(N)NDS solution per puff) and 15-s puff interval into a 1 L exposure chamber during the 20 min period, although exposure concentrations were not reported. After 1 week, mean MCC was not significantly different between unexposed (8.6%), PG-exposed (7.5%), or PG/nicotine-exposed (11.2%) mice. However, after 3 weeks, MCC was significantly higher in the PG-exposed animals (8.6%, 17.2%, and 8.7% in unexposed, PG-exposed, and PG/nicotine-exposed mice, respectively). Serum cotinine levels were significantly higher in mice exposed to PG/nicotine in comparison with control and PG-only exposure groups. No differences were seen in tracheal histology. The authors concluded that chronic exposure to an aerosol containing PG and nicotine slowed MCC in comparison with the rate associated with exposure to aerosol containing PG without nicotine.

72. Salturk et al. (2015) reported that of 8 female Wistar rats exposed to E(N)NDS aerosol (Ego T device with 0.9% nicotine[45], flow rate 200 mL/min), 60 min/day for 4 weeks, 2 developed hyperplasia and 4 metaplasia of the larynx. Rates in

[44] A 2-s, 35 mL puff every 10 s diluted with room air and provide at a flow rate of 10 L/min; further constituents not stated
[45] Other constituents not described

unexposed rats were 0/8 (hyperplasia) and 1/8 (metaplasia). However, statistical analysis did not indicate any significant differences between the two groups.

73. Analysis of BALF from female CD-1 mice exposed to E(N)NDS aerosol (50/50 PG/VG, 24 mg/mL nicotine), 60 min/day (9 s/min) for 4 weeks, by a 'respiratory tract only' system, showed no alterations in airway immune cell populations or histologic changes in the lung, but altered levels of some inflammatory cytokines as compared with air-exposed controls who underwent the same protocol. Serum levels of Pentraxin 3, an acute phase reactant in mice, were also increased in E(N)NDS aerosol-exposed mice in comparison with controls (Hwang et al. 2016).

74. Lim and Kim (2014) reported that exposure to E(N)NDS liquid can exacerbate allergy-induced asthma. Ovalbumin (OVA)-sensitised, female BALB/c mice were treated by intra-tracheal instillation of 100 µL of a 50-fold saline-diluted E(N)NDS liquid (purchased from Z-company, Korea, 16 mg/mL nicotine, other constituents not stated) twice per week for 10 weeks. Increased inflammatory cell infiltration to the airway and cytokine production, and OVA-specific IgE production were observed, as well as increased airways hyper-reactivity at higher levels of methacholine challenge, in treated compared with untreated mice.

75. Exposure to E(N)NDS aerosols led to impairments in pulmonary function, without pulmonary inflammation, in a mouse model designed to simulate effects of exposure during adolescence (Larcombe et al. 2017). Groups of 12 female BALB/c mice were whole-body exposed between the ages of 4-12 weeks to control air (AIR), CC smoke (SMOKE), or 1 of 4 E(N)NDS aerosols of 'American Tobacco' flavour, as follows: PG[46] (0-PG), PG + 12 mg/mL nicotine (12-PG), VG (0-VG[47]), or VG + 12 mg/mL nicotine (12-VG[48]). Average measured chamber concentrations were reported as 0.014 g/cm^{-3} for PG and 0.018 g/cm^{-3} for VG [49]. Mice were exposed for 1 h/day, 5 days/week from weeks 4-10, then twice daily for 1 h, 5 days/week, during weeks 11 and 12. After 8 weeks of exposure, all treatment groups weighed significantly less than AIR controls, with the lowest weight gains in nicotine-exposed mice[50]. Lung mechanics and function were assessed 24 h after the final exposure. Mice exposed to E(N)NDS aerosols showed various differences in pulmonary function compared with AIR controls, including decreased airway resistance at functional residual capacity (FRC) (0-PG and 0-VG), increased tissue damping at FRC (all 4 E(N)NDS groups), increased tissue elastance at FRC (0-PG and 0-VG), decreased lung volume and changes in volume dependence of tissue damping and elasticity (0-PG, 0-VG, 12-PG). Methacholine challenge tests showed that SMOKE- and VG-exposed mice were significantly more responsive than AIR- or PG-exposed

[46] PG-based aerosols contained 100% PG as the carrier.
[47] 95.3% VG / 4.70% PG
[48] 97.53% VG / 2.47% PG
[49] The Secretariat assumes that the concentrations should be reported as g/cm^3 . Exposure concentrations of 0.014 g/cm^3 and 0.018 g/cm^3 are equivalent to 14,000,000 mg/m^3 and 18,000,000 mg/m^3, respectively, which is far higher than other studies reported in this paper so itis possible that there is a reporting error.
[50] It was noted that there were differences in starting weights and individual weight gains.

mice, whether or not nicotine was present in the aerosol. SMOKE but not E(N)NDS exposure was associated with increased pulmonary inflammation (increased BAL cells). Overall, the authors concluded that 1] VG-based E(N)NDS aerosols induced more severe functional pulmonary impairments than PG-based aerosols, and 2] there was little effect of the presence or absence of nicotine.

76. Lee et al. (2018) measured nitrosamine-related DNA damage in lung, heart, liver, and bladder tissues of mice exposed to E(N)NDS aerosol for 12 weeks. Male Charles River mice (n=10/group) were exposed to aerosol from an NJOY tank-system (50/50 PG/VG, 10 mg/mL nicotine) in an exposure chamber, 3 h/day, 5 days/week, for 12 weeks. Aerosols were generated at 4.2 V, 1.96 A with a puff regime of 4-s, 35-mL puffs at 30-s intervals, mixed with filtered air before entering the 1 m^3 exposure chamber. Controls were exposed to filtered air. The presence of DNA adducts in genomic DNA isolated from tissues was evaluated by immunoblotting. O^6-methyl-deoxyguanosine (O^6-medG) adduct and the 1,N^2-propano-deoxyguanosine (PdG) adduct, γ-OH-PdG, were detected in lung, bladder and heart tissues, with higher levels determined in lung compared with bladder and heart tissues (reported as 2-8–fold higher for O^6-medG and 2-3–fold higher for γ-OH-PdG). A correlation between levels of the two adduct types in individual tissue samples was also reported. Analysis of lung tissues indicated that the aerosol exposure was also associated with significantly reduced DNA repair activity (nucleotide excision repair, NER, and base excision repair, BER), and reduced levels of NER- and BER-related proteins in the lung tissue. DNA parameters were inversely correlated with adduct levels. Taken together with the findings of additional studies in human cells *in vitro*, the authors suggested that nicotine nitrosation occurs *in vivo* in mice, thus exposure to E(N)NDS aerosol containing nicotine would be likely to be carcinogenic to the lung and bladder and harmful to the heart.

77. Werley et al. (2016) conducted a 90-day OECD-guideline study of the effects of exposure to E(N)NDS aerosol mixtures in rats. The full text of this publication is provided at Annex B. Groups of Sprague-Dawley rats were nose-only exposed to aerosols of vehicle control (77% PG, 23% glycerol), Formulation 1 (75.5% PG, 22.5% glycerol, 2% nicotine), or Formulation 2 (62.3% PG, 18.1% glycerol, 2% nicotine, 17.6% 'proprietary flavour') for 16, 48, or 160 min/day to achieve daily doses of 3.2, 9.6, or 32.0 mg/kg bw/day TPM, respectively, for periods of up to 90 days, followed by a 42-day recovery period. Particle mass median aerodynamic diameter (MMAD) values were in the range 1.1-1.3 µm (GSD, 1.54-1.63).

78. The authors reported that 'There were no treatment-related clinical observations. All clinical findings in the Formulation 1 and Formulation 2 treated groups were noted with similar incidence in the vehicle control group, or were common findings for laboratory rats of the same age and strain'. However, data were not shown. Plasma cotinine levels, measured at days 28 and 90, were reported to be 'proportional to the daily delivered dose progression', with high TPM exposures associated with approximately ten-fold higher levels than low TPM exposures. Values were not significantly different at days 28 and 90.

79. Lower body weight gains were associated with exposure to Formulation 1 and Formulation 2, compared with vehicle, and with higher TPM exposure compared with lower TPM exposure. Food consumption was reduced in Formulation 2 males for most of the 90-day exposure period, while treatment-related effects on food consumption were generally not observed in females.

80. At necropsy, the high TPM dose groups tended to have higher lung weights than low TPM dose groups (including significantly higher lung weight in the high compared with low TPM dose vehicle group), but these differences seemed to resolve after the recovery period. All high TPM exposures, i.e. vehicle-only or Formulation groups, were also associated with decreased serum albumin and increased serum phosphorus in males, and with increased AST (vehicle group) and serum phosphorus (Formulation 2 group) in females, compared with low TPM exposures, but these differences also resolved after recovery.

81. Clinical chemistry and cytology analyses of BALF at days 28, 90, and 132 indicated various changes, for example increased total protein and alkaline phosphatase (ALP), and higher levels of neutrophils and reduced proportions of alveolar macrophages, associated with high compared with lower TPM exposures. A detailed breakdown of all BALF clinical chemistry and cytology findings is shown in Tables 5 (28 days), 6 (90 days), and 7 (132 days) of the publication. In general, these changes appear to have resolved after the recovery period.

82. Histopathologic findings are summarised in Tables 9 and 10 of the publication. Histopathologic evaluation showed changes in the nose, lung, and larynx in all groups, including mild mucous cell hyperplasia in the nose, mild vacuolation of ciliated respiratory epithelium in the nose, dose-related increase in alveolar macrophages in the lungs, and non-dose-related mucin exudate in the larynx. Mucous cell hyperplasia in the nose persisted after recovery. Overall, the authors of this study determined the mid TPM dose (9.6 mg/kg bw/day) to be a no observed effect level (NOEL) for each treatment group, based on body weight decreases. These effects were greatest in high TPM Formulation groups, and authors suggested that suppressive effects of nicotine on appetite may have been involved.

83. Phillips et al. (2017) conducted a 90-day OECD TG 413 inhalation study of PG/VG mixtures at 3 test concentrations, without or with nicotine[51]. Groups of 10 male + 10 female Sprague-Dawley rats underwent nose-only exposure to nebulised test material as shown in Table 2 for 13 weeks (6 h/day, 5 days/week).

Table 2. Exposure groups in the 90-day inhalation study of Phillips et al. (2017).

Group	PG (mg/L)	VG (mg/L)	Nicotine (mg/L)	MMAD (µm)

[51] The full text of this publication is available at: https://www.sciencedirect.com/science/article/pii/S0278691517305112?via%3Dihub#mmc1 (accessed 18/06/18).

Sham (filtered air)	0	0	0	-
Vehicle (saline)	0	0	0	1.4
Low (PG/VG)	0.174	0.210	0	1.7
Med (PG/VG)	0.520	0.630	0	2.0
High (PG/VG)	1.520	1.890	0	2.0
Nicotine + Low (PG/VG)	0.174	0.210	0.023	1.8
Nicotine +Med (PG/VG)	0.520	0.630	0.023	2.0
Nicotine + High (PG/VG)	1.520	1.890	0.023	1.9

84. The authors calculated that the maximal exposure tested (group 'High (PG/VG)') would be a human equivalent dose (HED) for a 60 kg adult of 4.3 g/day PG and 5.3 g/day VG, corresponding to approximately 4 mL/day of a 100% solution of each compound. The maximum HED for nicotine was calculated as 66 mg, which would correspond to approximately 3.3 mL E(N)NDS liquid containing 20 mg/mL nicotine (the maximum allowed concentration in Europe).

85. Respiratory parameters (data are provided in Supplemental Figure 1 of the publication): Male rats in the 'High (PG/VG)' exposure group had significantly lower peak inspiratory flow compared with the saline-treated group and compared with the 'Nicotine + High (PG/VG)' group. Increases in respiratory parameters (peak inspiratory flow, tidal volume, minute volume) were seen in some of the nicotine-exposure groups, most prominently in female rats. Otherwise no clear pattern of changes was seen.

86. Food consumption and body weight (data are provided in Supplemental Figure 2 of the publication): Compared with the saline-exposed animals, food consumption was increased in association with nicotine exposure in both male and female rats (all PG/VG doses). In females, increased food consumption was seen in all nicotine exposure groups compared with non-nicotine exposure groups. On day 91 of the study, for males, body weight was slightly lower in the 'Nicotine + High (PG/VG)' compared with saline group. In females, a significantly higher body weight at day 91 was observed in all nicotine exposure groups compared with non-nicotine groups and also compared with the saline exposure group.

87. Lung weight and histopathology (data are provided in Supplemental Figure 3 and Figure 2 of the publication): In females, absolute lung weights were increased in all nicotine exposure groups compared with saline exposure, and in 'Nicotine + High (PG/VG)' compared with 'High (PG/VG)'. There were no significant differences between groups in normalised lung weight to body weight in females. In males, absolute lung weight was lower in 'High (PG/VG)' compared with saline. Normalised lung weight was lower in the sham group compared with saline, and higher in 'Nicotine + High (PG/VG)' compared with both the saline group and the 'High (PG/VG)' group. Histopathological examination showed some changes in the

respiratory tract, which the authors considered to be minimal adaptive changes (e.g. due to dehydration). The findings were mainly observed in the larynx, as follows. Compared with saline-exposed groups: basal cell hyperplasia in 'Nicotine + High (PG/VG)' females at the base of the epiglottis, and in 'Nicotine + Low (PG/VG)' females and 'Nicotine + High (PG/VG)' males at arythenoid projections; squamous metaplasia in 'Nicotine + High (PG/VG)' females and males at the base of the epiglottis and arythenoid projections, and in 'Nicotine + Med (PG/VG)' females at the base of the epiglottis. Compared with non-nicotine exposure (at the same PG/VG exposure level): increased squamous metaplasia at arythenoid projections in 'Nicotine + High (PG/VG) females and males. Compared with saline exposure, 'Low (PG/VG)' and 'Med (PG/VG)' exposures were associated with decreased infiltration of unpigmented macrophages in female rats, both with and without nicotine, but no effects were seen in male rats. BALF analysis indicated a slight increase in total cells (an indicator of inflammation) in 'Nicotine + High (PG/VG)' females compared with the equivalent non-nicotine group, but no differences were observed for any exposure groups in comparison with saline treatment.

88. Gene and protein expression profiling (data are provided in Supplemental Figure 6 and Figure 3 of the publication): Profiling was performed on nasal epithelium and lung of female rats (6 per group). As compared with saline controls, in nasal epithelium, treated groups showed no differences in gene expression. Seven proteins were downregulated in the 'High (PG/VG)' group, but this was not observed in the 'Nicotine + High (PG/VG)' group. In the lung, some gene expression changes were observed in the 'Low (PG/VG)' and 'Nicotine + Low (PG/VG)' groups, but these were not considered to be treatment related. Some altered gene sets were found to be associated with nicotine exposure, including upregulation of xenobiotic metabolising enzymes, Cyp1a1 and Fmo3, and downregulation of T-cell-related transcripts.

89. Haematology and clinical chemistry (data are provided in Supplementary Figure 7 and Figure 4 of the publication): Compared with saline treatment, effects on red blood cell parameters were seen in female (but not male) rats in the 'Nicotine + High (PG/VG)' group, which the authors suggested may be related to a stress response to nicotine exposure (also indicated by changes in thymus and adrenal gland weights). The presence of nicotine was also associated with some lower total cholesterol and lower glucose concentrations (males and females) and lower creatinine and calcium (females) compared with saline and/or equivalent-dose PG/VG-only groups.

90. Liver (data are provided in Figure 5 and Supplementary Table 4 of the publication): Exposures with nicotine were associated with effects on the liver (increased absolute and normalised liver weights, enzyme activity, hepatocyte vacuolation), with effects generally more pronounced in females than males. Effects were not seen in non-nicotine treatment groups, except for decreased alanine aminotransferase in 'High (PG/VG)' and sham-exposed males and increased alanine aminotransferase in sham-treated females, as compared with saline treatment

groups. These findings were supported by findings from gene-expression studies, indicating an association of nicotine exposure with lipid oxidation, gluconeogenesis, ketone body formation, and cholesterol biosynthesis. The authors noted that although hepatocyte vacuolation might be considered as an adverse effect, studies have suggested that it is in fact an adaptive response.

91. Overall, the authors considered that exposure to PG/VG aerosols showed minimal biological effects in comparison with exposure to nebulised saline, with no indication of toxicity. Inclusion of nicotine in the exposure led to effects that were consistent with findings from previous studies of nicotine, including upregulation of xenobiotic-metabolising enzymes in the lung and metabolic effects, such as reduced serum lipid concentrations and expression changes of hepatic metabolic enzymes. Authors concluded that 'No toxicologically relevant effects of PG/VG aerosols (up to 1.520 mg PG/L + 1.890 mg VG/L) were observed, and no adverse effects for PG/VG/nicotine were observed up to 438/544/6.6 mg/kg/day.'

92. Garcia-Arcos et al. (2016) conducted a study in which mice were whole-body exposed for 1 h/day to 0.4 mL of a 50:50 PG/VG commercial E(N)NDS liquid, with or without 18 mg/mL nicotine, or phosphate-buffered saline control, 5 days/week for 4 months. Mice exposed to nicotine-containing, but not nicotine-free, E(N)NDS liquid showed increased airway hyper-reactivity, inflammatory cell infiltration in the lung, airway enlargement, mucous production, and lung cell apoptosis. Similar findings were observed in human airways cells treated with E(N)NDS liquid with/without nicotine *in vitro*.

93. Crotty Alexander et al. (2018) suggested that repeated exposure to E(N)NDS aerosol may lead to increased inflammation, organ damage, and 'cardiorenal' and hepatic disease. In this study, C57BL/6 mice (noted by the authors to be susceptible to emphysema and oxidative stress) and CD-1 mice were exposed to E(N)NDS aerosol produced from an e-liquid consisting of 50% PG, 50% VG, and 24 mg/L nicotine. Aerosol was produced at 3.4 V and exposures were achieved using a nose-only system, flow rate of 2 L/min, exposure time 4 s per 20-s period, 60 min/day, 5 days/week, for 3 months (C57BL/6) or 6 months (CD-1). Controls were exposed to room air in the same manner as comparator test groups. One week before the end of the exposure, renal and cardiac function tests were performed, and following euthanasia blood and organs were collected. In addition, transcriptomic analyses of renal and cardiac tissues after 4 weeks of exposure were carried out.

94. In C57BL/6 mice, E(N)NDS aerosol exposure was associated with 20% reduced glomerular filtration rate[52], while increased collagen deposition (an indicator of renal fibrosis) was observed in both mouse strains exposed to E(N)NDS, in comparison with air-control groups. CD-1 mice had increased cardiac collagen-3 expression after 4 weeks E(N)NDS exposure, and higher liver collagen levels after 6 months E(N)NDS exposure, compared with air controls. E(N)NDS exposure was associated with changes in levels of circulating pro-inflammatory cytokines and pro-

[52] Evaluation of glomerular filtration rate in CD-1 mice was not reported

fibrotic proteins in both mouse strains. No pulmonary inflammation, emphysema, or fibrosis was evident in CD-1 mice[53], but alterations in BAL inflammatory cytokine profiles were observed.

95. Olfert et al. (2018) reported that chronic exposure to E(N)NDS aerosol accelerates aortic arterial stiffness, significantly impairs aortic arterial function, and may lead to impaired cardiac function. In this study, female C57BL/6J mice (n=15/group) were whole-body exposed to either E(N)NDS aerosol (cappuccino-flavoured, 18 mg/mL nicotine[54]; third generation eGrip OLED Joyetech device operated at 4.9 V), 3R4F reference CC smoke, or filtered air, for 8 months (4 x 1-h time blocks per day with 30 min intervals, 5 days/week). Average TPM in the E(N)NDS exposure chamber was 59 mg/m^3 (which the authors noted to be relatively low in comparison to CC smoke exposures used to induce chronic COPD in CC smoke chamber studies).

96. Arterial stiffness, measured by ultrasonography parameters, was not different between the groups at baseline or at 4.5 months, but showed a significant increase in the E(N)NDS (2.5-fold) and CC smoke (2.8-fold) exposure groups at 8 months, compared with controls. *Ex vivo* evaluation of aortic vessel constrictor response after 8 months showed reduced vasodilatory response to methacholine in both E(N)NDS (24% lower) and CC smoke (33% lower) groups compared with controls. Emphysema-type changes were noted on histological and respiratory function test examinations of CC smoke-exposed mice, but similar changes were not observed in the E(N)NDS exposure group.

6 Reproductive and developmental toxicity

97. Nicotine[55] crosses the placenta and nicotine exposure during pregnancy has the potential to adversely affect normal development of the fetus, particularly neurodevelopment. Animal studies have shown adverse effects of nicotine on fetal airway development and lung histology. Summaries of these data on the developmental toxicity of nicotine can be found in recent publications by U.S. Department of Health and Human Services (2016) and NAS (2018), also summarised in the accompanying paper, TOX/2018/25. In summarising the health risks of exclusive E(N)NDS use, a recent WHO report on 'Electronic Nicotine Delivery Systems and Electronic Non-Nicotine Delivery Systems (ENDS/ENNDS)' concluded that 'Foetal and adolescent nicotine exposure may have long-term consequences for brain development, potentially leading to learning and anxiety disorders. The evidence is sufficient to warn children and adolescents, pregnant women, and women of reproductive age against ENDS use and nicotine.' (WHO 2016).

[53] Evaluation of these endpoints was not reported for C57BL/6 mice
[54] Other constituents not stated
[55] The potential toxicity of nicotine in E(N)NDS is addressed in TOX/2018/25.

6.1 Humans

98. No epidemiological or clinical studies were identified relating to reproductive or developmental toxicity of E(N)N)DS use in humans.

6.2 Animal studies

99. Three studies were identified that evaluated reproductive and/or developmental toxicity of E(N)NDS aerosol mixtures, both with or without nicotine.

100. A study in mice of E(N)NDS aerosol exposure during gestation and early postnatal life indicated effects on subsequent behavioural parameters. Pregnant C57BL/6J mice were whole-body exposed to PG or PG/2.4% nicotine aerosol during gestation, and mothers + offspring were exposed from post-natal days 2-16. Exposures were achieved as 6-s puffs every 15 s into a 13.5 x 9 x 8.7 cm chamber, from a total of 600 µL liquid, over a period of approximately 20 min, once per day. Exposure concentrations were not reported. Behavioural tests at 14 weeks showed behavioural alterations in male offspring from the PG/2.4% nicotine group compared with room air- or PG-exposed male offspring, indicating effects of nicotine exposure on brain development. Mean pup weight at birth and throughout post-natal development was significantly lower in the PG group than the control or PG/nicotine groups. Mean pup weight in the PG/nicotine group was significantly lower than that of controls from post-natal day 7 onwards (Smith et al. 2015).

101. McGrath-Morrow et al. (2015) found that nicotine exposure from E(N)NDS aerosol affected alveolar growth and lung cell proliferation. Neonatal C57BL/6J mice were whole-body exposed to aerosol produced from 400 µL E(N)NDS liquid (PG or PG/1.8% nicotine; no flavouring) from days 1-10 after birth, once per day on days 1 and 2, then twice per day. Exposure was achieved from 6-s puffs every 15 s over approximately 20 min. Exposure concentrations were not reported. As compared with room air-exposed mice, total body weight was decreased in both the PG and PG/nicotine groups (by 11.5% and 13.3%, respectively). Exposure to PG/nicotine was associated with impaired alveolar cell proliferation compared with room air- and PG-only exposure, indicating an effect of nicotine on lung growth. There were no differences in markers of apoptosis of oxidative stress between the groups.

102. Chen et al. (2018) reported that maternal exposure to E(N)NDS aerosol, with or without nicotine, altered cytokine levels in both maternal and offspring lungs. Adult female Balb/c mice (numbers not reported[56]) were exposed to E(N)NDS aerosol (50/50 PG/VG, tobacco flavour), either with ('E-cig18') or without ('E-cig0') 18 mg/mL nicotine[57], for 15 min, twice per day with a 5-min interval, from 6 weeks before mating until pups were weaned at post-natal day 20. To achieve this, the adult females were removed from the home cage and placed in a 9 L exposure chamber. Adult male mice and offspring did not undergo exposures, but remained in the home

[56] Possibly 6/group
[57] Aerosol generated by KangerTech NEBOX, 4 x 5-s puffs at 30 W, 20-s interval

cage. Measured exposure concentrations were not reported. Control animals underwent the same procedure, but were exposed to room air instead of E(N)NDS aerosol. Male offspring were studied at postnatal day 1, postnatal day 20, and at 13 weeks. Mothers were studied when pups were weaned.

103. After the first 6 weeks of exposure, E-cig0 mice had only gained one-third of the weight of control mice, while weight gain in E-cig18 mice was not affected. There were no differences in weight gain between maternal groups at the end of the experiment, but retroperitoneal fat mass was reduced in both exposure groups compared with controls. Evaluation of lung cytokine levels showed increased IL1-β in E-cig18, increased IL-6 in E-cig0, and increased TNF-α in both E-cig0 and E-cig18, compared with controls.

104. In offspring, weight gain did not differ between groups at postnatal day 1. At postnatal day 20, E-cig0 offspring were significantly heavier and E-cig18 were significantly lighter than controls, liver weight was significantly increased in E-cig18 offspring, and both E-cig0 and E-cig18 offspring had significantly increased retroperitoneal fat mass, compared with controls. At 13 weeks, body weights did not differ between groups, but E-cig0 had reduced liver weight compared with the other two groups, and retroperitoneal fat mass was increased in both E-cig0 and E-cig18 groups compared with controls.

105. Altered lung gene expression levels of alveoli developmental markers were noted at post-natal day 20 in E-cig18 and E-cig0 offspring, compared with controls. At 13 weeks, IL1-β was increased and TNF-α was decreased in E-cig18 and E-cig0 offspring compared with controls, and these changes were correlated with changes in signalling-protein expression levels. Global DNA methylation in the lungs was increased three-fold in E-cig0 and two-fold in E-cig18 offspring at postnatal day 1, compared with controls. Authors postulated that as effects on lung markers in mothers and offspring were observed with exposure to E(N)NDS aerosol both with and without nicotine, the effects were likely to be due to 'by-products' of vaporisation rather than to nicotine.

106. Lauterstein et al. (2016) reported effects of exposure to E(N)NDS aerosol, both with and without nicotine. Pregnant C57BL/6J mice were whole-body exposed for 3 h/day, 5 days/week, throughout gestation, to room air or E(N)NDS aerosol[58] (blu, classic tobacco flavour), without or with 13-16 mg/mL nicotine[59]. Average particulate concentrations in the exposure chambers were 25.6 mg/m^3 and 30.7 mg/m^3 total suspended particulates, with and without nicotine, respectively. Pups received the same exposure from post-natal day 4-6 to 1 month of age, at which point brain frontal cortex tissue was subjected to transcriptomic analysis. There were no effects on birth weight or pup weight gain. Transcriptomic analyses indicated alterations in gene expression in the frontal cortex, including pathways associated with downstream adverse neurobiological outcomes, associated with

[58] Aerosol described as 73% PG and/or VG, 15% water, 11% flavourings, 1% nicotine
[59] 35 mL puff volume, 4-s puffs, 30-s intervals, mixed with filtered air.

E(N)NDS exposure both without or with nicotine, with the greatest effect seen in female offspring exposed to aerosol without nicotine.

107. In a follow-up report, the same group described evaluation of parameters of neuro-inflammation and neurotrophins. Experimental details were as reported for the study of Lauterstein et al. (2016) (paragraph 106). Exposure concentrations were reported as 25.6 mg/m^3 (with nicotine) and 30.7 mg/m^3 (without nicotine) total particulates. Reductions in hippocampal *Ngfr* and *Bdnf* gene expression, and in serum levels of cytokine 1L-1β, were observed in both exposure groups in comparison with controls exposed to filtered air, and non-nicotine-exposed mice had decreased serum levels of IL-2. Increased expression of Iba-1, a marker of microglia, was observed in the cornus ammonis 1 region of the hippocampus in male and female mice exposed to E(N)NDS without nicotine. Authors concluded that exposure to E(N)NDS aerosols, both with or without nicotine, poses a risk to the developing central nervous system (Zelikoff et al. 2018).

7 Genotoxicity and carcinogenicity

108. The cancer risk from E(N)NDS use would, in theory, be expected to be lower than that from CC smoking, for reasons including the absence of tobacco constituents, lower levels of nicotine-associated TSNAs, and lack of combustion. Nevertheless, other constituents such as flavourings are present in E(N)NDS liquids for which the mutagenic/carcinogenic effects on vaporisation and inhalation are uncertain. In addition, E(N)NDS aerosols have in some cases been reported to contain DNA-reactive substances such as formaldehyde (produced as a degradation product of E(N)NDS liquid solvents), and some studies have described cytotoxicity of E(N)NDS aerosols *in vitro* (*reviewed by* NAS (2018)).

109. Reports addressing the potential genotoxicity and/or carcinogenicity of E(N)NDS aerosols are not reviewed in detail here as these aspects are being considered for review by the Committee on Mutagenicity (COM) and the Committee on Carcinogenicity (COC). A brief overview is given below.

110. NAS (2018) noted that only one epidemiological study was identified that reported cancer incidence in relation to E(N)NDS use. Manzoli et al. (2017) conducted a prospective cohort study including 480 tobacco smokers, 229 E(N)NDS users, and 223 dual users, followed for 24 months. The primary outcome was abstinence from tobacco smoking, but self-reported 'serious adverse events' were also recorded, including one participant who reported 'lung cancer' (at baseline/24 months, a user of tobacco/tobacco), one 'blood cancer' (tobacco/quit), and 12 participants who reported 'Cancer, others'[60] (2 E(N)NDS/E(N)NDS, 3 E(N)NDS/tobacco, 1 E(N)NDS/quit, 3 dual/tobacco, 1 tobacco/E(N)NDS, and 2 tobacco/tobacco). Based on these data, NAS (2018) calculated a risk ratio for cancer from E(N)NDS use of 2.49 (95% CI 0.42-14.72) vs. tobacco cigarettes as the

[60] Described in a footnote as 'All other cancers excluding cancer of the larynx, lung, esophagus, pancreas, cervix, kidney, bladder, and hematological.'

referent, noting that the results 'do not provide any indication for cancer risk reduction from sole use of e-cigarettes'. However, the data were noted to be extremely limited and of low quality.

111. No data were identified from animal studies on the potential carcinogenicity of long-term exposure to E(N)NDS aerosols. A recent report by Lee et al. (2018) noted that 12-week exposure of mice to E(N)NDS aerosol containing nicotine led to nicotine-derived DNA adduct formation in lung, bladder, and heart tissues, and thus such exposure could be carcinogenic (this study is described in paragraph 76)

112. In Chapter 10 'Cancers' of the report on the 'Public Health Consequences of E-cigarettes', the NAS (2018) committee summarised their findings as follows:

> "**Finding**: There are no available epidemiological studies on the potential association between e-cigarette use and cancer in humans to make any conclusions. This holds true for comparisons of e-cigarette use compared with combustible tobacco cigarettes and e-cigarette use compared with no use of tobacco products.
>
> *Conclusion 10-1. There is **no available evidence** whether or not e-cigarette use is associated with intermediate cancer endpoints in humans. This holds true for comparisons of e-cigarette use compared with combustible tobacco cigarettes and e-cigarette use compared with no use of tobacco products.*
>
> *Conclusion 10-2. There is **limited evidence** from in vivo animal studies using intermediate biomarkers of cancer to support the hypothesis that long-term e-cigarette use could increase the risk of cancer; there is **no available evidence** from adequate long-term animal bioassays of e-cigarette aerosol exposures to inform cancer risk.*
>
> *Conclusion 10-3. There is **limited evidence** that e-cigarette aerosol can be mutagenic or cause DNA damage in humans, animal models, and human cells in culture.*
>
> *Conclusion 10-4. There is **substantial evidence** that some chemicals present in e-cigarette aerosols (e.g., formaldehyde, acrolein) are capable of causing DNA damage and mutagenesis. This supports the biological plausibility that long-term exposure to e-cigarette aerosols could increase risk of cancer and adverse reproductive outcomes. Whether or not the levels of exposure are high enough to contribute to human carcinogenesis remains to be determined.*
>
> While evidence in humans for associations between e-cigarette use and cancer is extremely sparse, more abundant data have been generated in the in vitro and in vivo setting, including some positive studies and some negative studies on mutagenesis of e-cigarette components. Due to the mixed results across different experimental conditions and for different outcomes, clear, consistent signals have yet to be observed."

8 Summary

113. Some clinical case reports have described adverse conditions that appear to have an onset coincident with the initiation of E(N)NDS use. These are mostly cases of non-infective upper or lower respiratory tract conditions, such as organising, eosinophilic, or lipoid pneumonias, bronchiolitis and interstitial lung disease. One report described post-surgical skin flap necrosis in a patient using E(N)NDS during the peri-operative period, and the authors cautioned that this may have been related to nicotine exposure. A small number of case reports described improvements in medical conditions associated with E(N)NDS use. In one case, ulcerative colitis, which onset in an adult male 4 weeks after he quit CC smoking, resolved when the patient subsequently took up using E(N)NDS (nicotine content was not noted). In another case, an adult male CC smoker with chronic idiopathic neutrophilia and hyperlipidaemia had normalisation of leucocyte and C-reactive protein on starting E(N)NDS (with nicotine) use and quitting CC smoking. The third case report described a young adult female non- (never) CC smoker with frequent episodes of tonsillitis and recurrent tonsilloliths, for whom these symptoms resolved 3 months after taking up E(N)NDS use (with 0-3 mg/mL nicotine).

114. Exposure to E(N)NDS aerosol has the potential, in theory, to damage the respiratory system or to exacerbate pre-existing lung disease. NAS (2018) discussed a variety of possible mechanisms by which this might occur, including a potential impact of nicotine on host-defence mechanisms in the lungs and immune response, cough reflex and MCC, and the potential effects of other constituents (ultrafine particles, oxidants, flavourings and other chemicals) to damage the airways and lung parenchyma and to cause inflammation and broncho-constriction, leading to airway remodelling and/or asthma, and ultimately to COPD and end-stage lung disease.

115. There is currently a limited data-set regarding the effects of long-term exposures to E(N)NDS on the respiratory system in humans. In addition, interpretation of the data is complicated by such factors as the wide variation in test product constituents, test procedures, subject characteristics, and delivery systems used. Many studies have focussed on the potential reduction in harm associated with E(N)NDS use in comparison with CC smoking and/or on effects of E(N)NDS exposure on short-term endpoints, such as respiratory function parameters and self-reported respiratory symptoms.

116. Acute respiratory effects of exposure to E(N)NDS aerosols has been assessed in CC smokers, ex-CC smokers, and non- or never CC smokers. Some studies have observed alterations in lung function parameters and decreased exhaled NO fraction, although other studies have reported no significant effects. One study observed that exposure of dual CC smokers/E(N)NDS users to E(N)NDS aerosol containing nicotine caused more severe effects on respiratory function in asthmatic compared with non-asthmatic subjects (Lappas et al. 2018). However, Boulay et al. (2017) found that exposure of asthmatic and non-asthmatic non-smokers to a PG/VG-only aerosol did not affect respiratory function parameters.

Exposure to E(N)NDS aerosol containing nicotine was observed to inhibit cough reflex, but this effect was not observed when a non-nicotine product was tested (Dicpinigaitis et al. 2016).

117. A group of researchers in Italy has published a number of studies, based on relatively small study populations, reporting improvements in lung function and pre-existing lung conditions such as asthma in CC smokers using E(N)NDS as an aid to quit smoking. Conversely, a larger study in the UK following CC smokers using E(N)NDS to aid quitting smoking reported a negative impact on lung function parameters over time, although the authors did not consider these changes to be clinically significant, suggesting that factors such as aging may be the cause (Walele et al. 2018). In one study, use of E(N)NDS to quit smoking was associated with negative effects on sino-nasal symptoms and MCC at 3 months as compared with subjects attempting to quit CC smoking without using E(N)NDS (Kumral et al. 2016).

118. A number of cross-sectional epidemiological studies (in California, Florida, Hawaii, Korea, Hong Kong) have reported significant associations of E(N)NDS use by adolescents with asthma or respiratory symptoms. These studies have been based on data obtained from large-scale surveys, using self-reported data on factors such as E(N)NDS use and CC smoking frequency, asthma diagnosis, and the presence of other respiratory symptoms. Studies reported significantly higher prevalence of asthma and/or other respiratory symptoms in E(N)NDS users compared with non-users, with these associations seen in both CC-smoking and non-CC-smoking E(N)NDS users.

119. Two studies reported proteomic analysis comparisons of samples (sputum, brush biopsies and BAL samples) obtained from airways of E(N)NDS users, CC smokers, and non-smokers, describing alterations specific to E(N)NDS use and also overlapping with CC use (Reidel et al. 2018, Ghosh et al. 2018).

120. A few studies have evaluated periodontal health in E(N)NDS users, either in the context of comparison with CC smokers, or evaluations of CC smokers switching to E(N)NDS use, including both positive (improvement in periodontal health) and negative (increased gingival inflammation) findings reported. One cross-sectional epidemiological study in Korea observed adverse effects associated with E(N)NDS use by adolescents on some of the oral health parameters evaluated: tongue and/or cheek pain was more highly associated with the use of non-nicotine-containing E(N)NDS, while cracked teeth was more associated with the use of nicotine-containing E(N)NDS) (Cho 2017).

121. Some of the constituents in E(N)NDS aerosols have the theoretical potential to be associated with the development of CVD, including ultrafine particles, metal particulates, and nicotine. However, there is currently a paucity of epidemiological data to address this aspect. The NAS (2018) report highlighted the requirement for studies assessing clinical (coronary heart disease, stroke, atherosclerotic peripheral artery disease) and sub-clinical (atherosclerosis, carotid intima media) endpoints in relation to E(N)NDS exposures as a major research need, concluding that there is

currently no available evidence as to whether E(N)NDS use is associated with clinical CVD outcomes.

122. A number of clinical studies have investigated acute cardiovascular effects of E(N)NDS exposures. In general, these studies have indicated effects such as increased heart rate, blood pressure that are related to the presence and bioavailability of nicotine in E(N)NDS aerosol. One study found that exposure to 10 puffs of E(N)NDS aerosol containing nicotine over a 10-min period was associated with increased endothelial progenitor cells in blood at 1 h post exposure (Antoniewicz et al. 2016) and another study reported increased markers of oxidative stress after 9 puffs of E(N)NDS containing nicotine in both smoker and non-smoker test groups (Carnevale et al. 2016).

123. Regarding cardiovascular effects of longer-term E(N)NDS aerosol exposures, two studies reported reduced blood pressure in hypertensive CC smokers switching to E(N)NDS in an attempt to quit (Polosa et al. 2016a, Farsalinos et al. 2016), while Moheimani et al. (2017b) reported that regular E(N)NDS use was associated with a shift in cardiac autonomic balance towards sympathetic predominance.

124. Repeat-dose animal studies have mostly investigated effects of E(N)NDS aerosols on the respiratory system, and have reported effects on the respiratory epithelium, lung inflammation and oxidative stress, and host-defence against respiratory pathogens and effects on lung development. A small number of studies have reported effects other than respiratory, including indicators of fibrosis in kidney, heart, and liver of mice and increased liver weight, enzyme activity and hepatocyte vacuolation in rats exposed to nicotine-containing E(N)NDS aerosol. One study identified nicotine-associated DNA adduct formation in lung, heart, and bladder tissues of mice exposed to nicotine-containing E(N)NDS for 12 weeks (Lee et al. 2018).

125. Nicotine is an established developmental toxin and the small number of animal studies that were identified showed potential developmental effects of exposure to nicotine-containing E(N)NDS aerosols. A recent World Health Organization (WHO) report on ‘Electronic Nicotine Delivery Systems and Electronic Non-Nicotine Delivery Systems (ENDS/ENNDS)’ concluded that ‘Foetal and adolescent nicotine exposure may have long-term consequences for brain development, potentially leading to learning and anxiety disorders. The evidence is sufficient to warn children and adolescents, pregnant women, and women of reproductive age against ENDS use and nicotine.’ (WHO 2016). Some studies have shown developmental effects of exposure to E(N)NDS aerosols without nicotine, including altered gene expression in lung (Chen et al. 2018) and brain (Lauterstein et al. 2016, Zelikoff et al. 2018).

126. Cancer risk from E(N)NDS use would be expected to be lower than that from CC smoking, for reasons including the absence of tobacco constituents, lower levels of nicotine-associated TSNAs, and lack of combustion (NAS 2018). Nevertheless, other constituents are present in E(N)NDS liquids for which the mutagenic/

carcinogenic effects on vaporisation and inhalation are undetermined. In addition, the presence of DNA-reactive substances (e.g. formaldehyde produced as a degradation product of e-liquid solvents) in E(N)NDS aerosols has been described in some reports, and some studies have described cytotoxicity of E(N)NDS aerosols *in vitro* (*reviewed by* NAS (2018)). A full review of studies relating to the genotoxicity and carcinogenicity of E(N)NDS was not carried out as these aspects will be considered by the Committees on Mutagenicity and Carcinogenicity, respectively. However, NAS (2018) noted that there is no available epidemiological or intermediate endpoint evidence in humans or from adequate long-term animal bioassays, limited evidence from *in vivo* animal studies using intermediate biomarkers, limited evidence that E(N)NDS aerosol can be mutagenic or cause DNA damage in humans, animal models and human cells in culture, and substantial evidence that some chemicals present in E(N)NDS aerosols (e.g. formaldehyde, acrolein) are capable of causing DNA damage and mutagenesis, and that this supports the biological plausibility that long-term exposure to E(N)NDS aerosols could increase risk of cancer and adverse reproductive outcomes. There is a question as to whether exposures are high enough to be of concern for human carcinogenesis.

9 Questions for the Committee

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

i. Does the Committee want further information to be provided on any of the studies described in this discussion paper?

ii. Is the Committee able to draw any conclusions from the data presented on the absolute risks of E(N)NDS use, with or without nicotine, or risks relative to smoking conventional cigarettes?

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
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Abbreviations

8-iso-PGF2a	8-iso-prostaglandin F2a
ALP	Alkaline phosphatase
AOR	Adjusted odds ratio
BAL(F)	Broncho-alveolar lavage (fluid)
BER	Base excision repair
CC	Conventional cigarette
CFTR	Cystic fibrosis transmembrane conductance regulator
CI	Confidence interval
COC	Committee on Carcinogenicity
COM	Committee on Mutagenicity
COPD	Chronic obstructive pulmonary disease
CVD	Cardiovascular disease
ECG	Electrocardiogram
E(N)NDS	Electronic nicotine delivery system(s) and electronic non-nicotine delivery system(s)
FEF_{25}	Forced expiratory flow at 25% of FVC
FEV_1	Forced expiratory volume in 1 s
FRC	Functional residual capacity
FVC	Forced vital capacity
HED	Human equivalent dose
HYRBS	Hawaii Youth Risk Behavior Survey
IL	Interleukin
KYRBWS	Korean Youth Risk Behavior Web-based Survey
LDH	Lactate dehydrogenase
LED	Light-emitting diode
MCC	Mucociliary clearance
MCP	Monochemoattractant protein
MMAD	Mass median aerodynamic diameter
MUC5AC	Mucin 5AC, Oligomeric Mucus/Gel-Forming
NAS	U.S. National Academy of Sciences
NER	Nucleotide excision repair
NET	Neutrophil extracellular trap
NO	Nitric oxide
NOAEL	No observed adverse effect level
OR	Odds ratio
OVA	Ovalbumin
PAH	Polycyclic aromatic hydrocarbon
PdG	Propano-deoxyguanosine
PEF	Peak expiratory flow
PG	Propylene glycol
SNOT-22	Sino-nasal outcome test
sNOX2-dp	Soluble NOX2-derived peptide
STIM1	Stromal interaction molecule-1
TNF-α	Tumour necrosis factor-α

TPM	Total particulate mass
TSNA	Tobacco-specific nitrosamine
VAMP8	Vesicle Associated Membrane Protein 8
VG	Vegetable glycerin(e) (glycerol)
VOC	Volatile organic compound
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 4: Toxicological and epidemiological evaluations of E(N)NDS aerosol exposures.

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

Relevant literature was obtained from reviews published by authoritative bodies, as described in paragraph 4 of the main report. In addition, searches for further literature relating to toxicity of E(N)NDS aerosol were identified as described below.

The following three sets of literature searches were performed by NCET at WRc/IEH-C under contract to PHE on 06/04/18 in Scopus and PubMed, with no limit of publication date.

Search 1

Scopus

(TITLE-ABS-KEY ("e-cig*" OR "electronic cigarette*" OR "electronic nicotine delivery system*") AND TITLE-ABS-KEY (*toxic* OR "health effect*" OR teratogen*)) AND (EXCLUDE (DOCTYPE , "bk")) AND (EXCLUDE (LANGUAGE , "French") OR EXCLUDE (LANGUAGE , "German") OR EXCLUDE (LANGUAGE , "Polish") OR EXCLUDE (LANGUAGE , "Italian") OR EXCLUDE (LANGUAGE , "Spanish") OR EXCLUDE (LANGUAGE , "Chinese") OR EXCLUDE (LANGUAGE , "Czech") OR EXCLUDE (LANGUAGE , "Croatian") OR EXCLUDE (LANGUAGE , "Danish") OR EXCLUDE (LANGUAGE , "Dutch") OR EXCLUDE (LANGUAGE , "Japanese") OR EXCLUDE (LANGUAGE , "Portuguese")): 539 refs

PubMed

((((e-cig* [Title/Abstract] OR "electronic cigarette*" [Title/Abstract] OR "electronic nicotine delivery system*"[Title/Abstract])) AND (*toxic* [Title/Abstract] OR "health effect*" [Title/Abstract] OR teratogen* [Title/Abstract]))) AND english[Language]: 252 refs

Search 2

Scopus

(TITLE-ABS-KEY ("e-cig*" OR "electronic cigarette*" OR "electronic nicotine delivery system*") AND TITLE-ABS-KEY (pulmonary OR lung OR "respirat*")) AND (EXCLUDE (LANGUAGE , "German") OR EXCLUDE (LANGUAGE , "French") OR EXCLUDE (LANGUAGE , "Spanish") OR EXCLUDE (LANGUAGE , "Italian") OR EXCLUDE (LANGUAGE , "Polish") OR EXCLUDE (

LANGUAGE , "Portuguese") OR EXCLUDE (LANGUAGE , "Chinese") OR EXCLUDE (LANGUAGE , "Czech") OR EXCLUDE (LANGUAGE , "Danish") OR EXCLUDE (LANGUAGE , "Greek") OR EXCLUDE (LANGUAGE , "Japanese") OR EXCLUDE (LANGUAGE , "Swedish")): 425 refs

PubMed

((((e-cig* [Title/Abstract] OR "electronic cigarette*" [Title/Abstract] OR "electronic nicotine delivery system*"[Title/Abstract])) AND (pulmonary[Title/Abstract] OR lung [Title/Abstract] OR respirat* [Title/Abstract]))) AND english[Language]: 195 refs

Search 3

Scopus

(TITLE-ABS-KEY ("e-cig*" OR "electronic cigarette*" OR "electronic nicotine delivery system*") AND TITLE-ABS-KEY (cardiovascular OR heart OR cardiac OR "blood pressure")) AND (EXCLUDE (DOCTYPE , "cr ") OR EXCLUDE (DOCTYPE , "bk ")) AND (EXCLUDE (LANGUAGE , "French ") OR EXCLUDE (LANGUAGE , "German ") OR EXCLUDE (LANGUAGE , "Polish ") OR EXCLUDE (LANGUAGE , "Czech ") OR EXCLUDE (LANGUAGE , "Dutch ") OR EXCLUDE (LANGUAGE , "Italian ") OR EXCLUDE (LANGUAGE , "Portuguese ") OR EXCLUDE (LANGUAGE , "Serbian ") OR EXCLUDE (LANGUAGE , "Spanish "))): 249 refs

PubMed

((((e-cig* [Title/Abstract] OR "electronic cigarette*" [Title/Abstract] OR "electronic nicotine delivery system*"[Title/Abstract])) AND (cardiovascular [Title/Abstract] OR heart [Title/Abstract] OR cardiac [Title/Abstract] OR "blood pressure" [Title/Abstract]))) AND english[Language]: 101 refs

For completion, the reference lists of selected papers were examined for further relevant publications, and additional *ad hoc* searches were carried out as considered appropriate.

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat
June 2018

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 4: Toxicological and epidemiological evaluations of E(N)NDS aerosol exposures.

Full text and supplementary material to Werley et al. (2016) reference

Werley, M. S., D. J. Kirkpatrick, M. J. Oldham, A. M. Jerome, T. B. Langston, P. D. Lilly, D. C. Smith & W. J. McKinney, Jr. (2016) Toxicological assessment of a prototype e-cigaret device and three flavor formulations: A 90-day inhalation study in rats. Inhalation toxicology, 28, 22-38.

This paper and associated supplementary material is attached. It is not being made publicly available for copyright reasons.

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