TOX/2018/25

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-cigarettes). Paper 5: Preliminary overview of nicotine toxicity

Background

1. The COT is reviewing the potential human health effects of electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (which, overall, may also referred to as 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, and a paper reviewing published data on the toxicity of E(N)NDS aerosols (TOX/2018/24) will be presented alongside this one at the July 2018 COT meeting.

2. This present paper gives an outline of knowledge on the toxicity of nicotine, which is a common constituent of E(N)NDS liquids. Due to restrictions of time, the paper is an overview of data from recently published reviews that have discussed nicotine toxicity in the context of exposure from E(N)NDS. Original publications have not been obtained or evaluated.

Introduction

3. E(N)NDS are battery-powered devices containing a liquid (E(N)NDS liquid or ‘e-liquid’). The E(N)NDS liquid is heated on use to produce an aerosol that is inhaled by the user (‘puffing’, ‘vaping’). E(N)NDS were first introduced commercially in China in 2004 and subsequently in the EU (2005) and USA (2007) as nicotine-delivery devices. The main constituent parts of an E(N)NDS device are a mouthpiece, cartridge (tank) containing E(N)NDS liquid, a heating element/atomizer, a microprocessor, a battery, and sometimes an LED light. Commercially available devices are sometimes categorised as first, second, or third generation. First-generation devices look like conventional cigarettes 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.

4. Constituents that have been identified in E(N)NDS liquids and/or aerosols include propylene glycol (PG), vegetable glycerine (VG, glycerol), 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.

5. The following sections summarise relevant narrative taken from three recent publications that have addressed the potential toxicity of nicotine in relation to exposure from ENDS. A link to the full-text version of the review article is given at the beginning each overview section. Original citations from the review articles are listed where they may be useful for cross-referencing, but these original publications were not retrieved or reviewed directly. Full details of the citations can be found in the reference lists of the review articles.

Royal College of Physicians (RCP, 2016)

3. In 2016, the Tobacco Advisory Group of the Royal College of Physicians published a report updating the use of harm reduction in tobacco smoking, including all non-tobacco nicotine products, and with a particular focus on ENDS. The full text of this report, entitled 'Nicotine Without Smoke: Tobacco Harm Reduction. A report by the Tobacco Advisory Group of the Royal College of Physicians', is available at: https://www.rcplondon.ac.uk/projects/outputs/nicotine-without-smoke-tobacco-harm-reduction-0 (accessed 17/05/18).

4. Chapter 4 of the report reviews the pharmacology and pathophysiology of nicotine.

5. Section 4.1 discusses the chemistry and absorption of nicotine. Only non-ionised nicotine can cross biological membranes and be absorbed into the bloodstream. As nicotine is a weak base with a pKa of approximately 8, absorption from the normally acidic medium of conventional cigarette (CC) smoke can be increased by increasing the pH. Inhalation of nicotine via smoking a CC leads to a rapid increase in arterial nicotine concentration, with peak values occurring around 20-30 s after exposure. Maximum achieved levels can be modified by smoking

behaviour. Arterial nicotine concentrations from CC smoking are higher than venous concentrations, and the rapid rate at which nicotine reaches the brain via the arterial blood underlies its highly addictive potential (see Fig 4.1 on page 53 of the report). Nicotine that is swallowed is absorbed from the gastrointestinal tract into the portal veins and undergoes substantial first-pass metabolism in the liver, thus concentrations in venous and arterial blood from this source are low. Conventional nicotine replacement therapy (NRT) products deliver nicotine via the skin, mouth or nose, avoiding first-pass metabolism. Thus, although achieved blood levels are higher than via oral exposure, use of NRT products leads to similar arterial and venous nicotine levels, which are much lower than the arterial levels achieved by inhalation. Nasal sprays deliver nicotine relatively quickly in comparison with other forms of NRT (see Fig 4.2 on page 54 of the report).

6. Approximately 70-80% of nicotine is metabolised to cotinine (Section 4.2), of which 90% is mediated by hepatic cytochrome P450 (CYP) 2A6 enzyme. Cotinine is then metabolised exclusively by CYP2A6 to 3'-hydroxycotinine. Nicotine and metabolites are excreted in the urine. The nicotine metabolite ratio (NMR), 3'-hydroxycotinine:cotinine, is an indicator of CYP2A6 nicotine clearance. CYP2A6 genotypic variation is associated with variable rates of nicotine metabolism, with slow metabolism associated with higher rates of abstinence from CC smoking and higher success rates of quitting, either unaided or with NRT. Other enzymes that have a minor role in nicotine metabolism include flavin-containing monooxygenase (FMO) 3, uridine diphosphate glucuronyltransferase (UGT) 2B10 and UGT2B17, which also show polymorphic variation.

7. The physiologic and pharmacologic effects of nicotine (Section 4.3) are mediated via binding to nicotinic acetylcholine receptors (nAChRs), which are expressed throughout the body, including the central nervous system and peripheral nervous system. nAChRs are ligand-gated ion channels comprising differing combinations of five transmembrane subunit proteins, α_2-α_{10} and β_2-β_4 subunits around a central pore. Receptor subtypes differ in aspects such as nicotine affinity, sensitivity to upregulation, and desensitisation. The expression of different subtypes shows a pattern of distribution within the brain, with $\alpha_4\beta_2$ being the most common. Tolerance to the stimulant effects of nicotine develops rapidly (days), leading to withdrawal symptoms, and chronic exposure leads to neuroadaptations, including desensitisation and upregulated expression of nAChRs. Binding of nicotine to nAChRs leads to the release of other neurotransmitters, including dopamine, serotonin, γ-aminobutyric acid (GABA), glutamate, noradrenaline, acetylcholine, and endorphins. Pairing of reward/reinforcing effects (e.g. dopamine release) with CC smoking-related environmental stimuli is thought to be linked to the development and maintenance of smoking-related cravings and relapse.

8. The toxicity of nicotine is discussed in Section 4.4. Ingestion of high doses can be fatal, but overdose by other routes of exposure is considered to be extremely unlikely. The LD_{50} in humans has historically been considered to be approximately 0.8 mg/kg bw, but has recently been re-evaluated to be 6.5-13 mg/kg bw (*cited ref.*

48: Mayer, 2014). Nicotine is not toxic at doses normally used in NRT in the short term, with NRT products considered to be safe for use over several weeks with no evidence of increased risk of heart attack, stroke, or death (*cited ref. 51*: Stead et al., 2012). However, there is a lack of data relating to long-term exposure to nicotine or NRT. Animal studies indicate that chronic exposure to nicotine itself is not carcinogenic (*cited ref. 53*: Hecht, 2003). *In vivo* and *in vitro* studies indicate tumour-promoting effects via aspects such as cell proliferation, angiogenesis, and apoptosis, although studies were carried out using high nicotine doses (*cited ref 37*: Schuller, 2009; *cited ref. 49*: Shields, 2011; *cited ref. 54*: Maier et al., 2011). Studies *in vitro* have shown negative effects of nicotine on the function of some cells of the cardiovascular system (*cited ref. 55*: Balakumar and Kaur, 2009) and on glucose metabolism (*cited ref. 56*: Bruin et al., 2008). In contrast, a five-year study of subjects who used NRT for several months or longer found no evidence of associations with cancer (lung, gastrointestinal, any) or cardiovascular disease (*cited refs. 57, 58*: Murray et al., 1996, 2009), and a clinical trial of NRT treatment for 8, 24, or 52 weeks did not identify adverse effects associated with differing exposure durations (*cited ref. 59*: Schnoll et al., 2015). However, no long-term data are available regarding the toxicity/safety of nicotine by inhalation exposure, other than data relating to CC smoking. Pragmatically, nicotine inhalation is considered to be relatively much less toxic than CC smoking, and nicotine is licensed by the UK Medicines and Healthcare products Regulatory Agency 'for use as a substitute or partial substitute for smoking tobacco, both for those attempting to quit and those not currently intending to make an attempt to quit, without any restriction on its duration of use' (*cited ref. 61*: Medicines and Healthcare Products Regulatory Agency, 2010). Findings from animal studies have indicated that nicotine has adverse effects on the developing fetus and on both fetal and adolescent cognitive development, but translation of these findings to the situation in humans in currently unclear (*cited ref. 63*: Bruin et al., 2010; *cited ref. 66*: Goriounova et al., 2012; *cited ref. 65*: Royal College of Physicians, 2013; *cited ref. 64*: US Department of Health and Human Services, 2014).

9. Sections 4.5-4.8 of the report discuss factors affecting nicotine addiction, noting that addictivity is strongly associated with speed of delivery to the brain and the rate at which nicotine is metabolised. The half-life for elimination is around 2 h. Rapid delivery of a high dose is associated with greater potential for addiction (see Fig. 4.3 on page 60). Section 4.6 notes other agents that are added to CC or present in CC smoke which enhance nicotine delivery and absorption, including sugars and polysaccharides to increase the formation of aldehydes, which can be addictive (e.g. acetaldehyde) or enhance the addictive potential of nicotine; menthol which increases palatability, facilitates deeper inhalation, and inhibits CYP2A6 activity; cocoa and chocolate, which contain theobromine, a bronchodilator; other flavourings; alkaline additives to optimise pH for nicotine delivery; and monoamine oxidase (MAO) inhibitors, which increase levels of amines in the brain.

U.S. Department of Health and Human Services (2016)

10. In 2016, the U.S. Surgeon General reviewed the scientific literature on current use, health consequences, and marketing of e-cigarettes, in the context of adolescents (11-13 and 14-17 years) and young adults (18-24 years). The full text of this report, entitled 'E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General', is available at: https://www.surgeongeneral.gov/library/2016ecigarettes/index.html (accessed 17/05/18).

11. Chapter 3, 'Health Effects of E-Cigarette Use Among U.S. Youth and Young Adults', documents evidence relating to health effects of ENDS aerosols and their constituents, with a focus on knowledge about the potential health effects of nicotine.

Dose and effects of inhaling aerosolized nicotine

12. Factors that affect the amount of aerosolized nicotine available for inhalation are discussed. These include the nicotine concentration in the e-liquid, power (voltage and resistance) of the ENDS device, and user behaviour (puffing topography). Plasma nicotine concentrations measured after ENDS use can vary substantially, with levels observed ranging from lower to higher than those achieved by CC smoking. There is variation across products, with cigalikes generally delivering less nicotine than tank systems, and low-resistance, dual-coil cartomizers being able to deliver nicotine at lesser or greater levels than CC, depending on the e-liquid nicotine concentration. In controlled sessions using equivalent types of devices and e-liquid nicotine concentrations, user puffing topography has been shown to affect nicotine delivery. One study showed a ten-fold variation in the plasma nicotine concentration (0.8-8.5 ng/mL) achieved across participants under equivalent conditions. ENDS users are reported to develop the capability to extract more nicotine from the devices as they become more experienced with use.

Aerosolised nicotine and cardiovascular function

13. Acute nicotine administration in humans leads to dose- and route-dependent effects on the cardiovascular system in adults, including increased heart-rate and blood pressure (BP), higher cardiac output, and increased myocardial oxygen demand (*reviewed by cited ref*: USDHHS, 2014). Animal studies have indicated negative effects on the cardiovascular system, including the production of inflammatory mediators involved in atherosclerotic pathogenesis (*cited ref:* Lau and Baldus, 2006), and induction of C-reactive protein (CRP) in macrophages (*cited ref:* Mao et al., 2012). Data on the effects of longer term exposure of humans to nicotine in ENDS are sparse but short-term studies have shown that measurable plasma nicotine increases in association with ENDS use leads to increased heart rate[1]. Reduced risk of cardiovascular disease has been reported in CC smokers switching to NRT but, as most quitting CC smokers use NRT for periods of around two years

[1] These studies are reviewed in the discussion paper, TOX/2018/24

or less, potential adverse effects in the longer term have not been determined. Studies in Scandinavian countries on users of Swedish-type moist snuff ('snus'), which has a high nicotine content and low tobacco-specific nitrosamine (TSNA) content, have shown no increased risk of myocardial infarction in non-CC-smoking snus users followed for periods of 4-29 years (*cited ref:* Hansson et al., 2012).

Aerosolized nicotine and dependence

14. Use of ENDS can deliver levels of nicotine to the blood that are, in some cases, comparable to or higher than those from smoking CC, which would allow nicotine dependence to develop and to be maintained. Consequently, some studies have reported rapid development of nicotine dependence in ENDS users. This section of narrative reviews knowledge to date, concluding that 'the addictive liability of e-cigarettes has the potential to be at least equivalent to that of conventional cigarettes, given the nicotine dose levels produced by these products, particularly among experienced users operating new-generation devices'.

Effects of nicotine in youth users and during pregnancy

15. There is substantial evidence that nicotine can negatively affect adolescent and fetal brain development (*cited ref:* reviewed by USDHHS, 2014). Limited epidemiological data are available, but animal studies have provided evidence of neuroteratogenicity and neurotoxic effects on developing adolescent brain. Rodent models are described as appropriate for studying the neurobiology of brain development in human teenagers. Expression and functional activity of acetylcholine receptors (AChRs) is higher in the adolescent forebrain compared with adult rodent forebrain, and studies have shown increased sensitivity to the rewarding effects of very low doses of nicotine in adolescent rats, as well as decreased levels of aversion/negative effects of short-term nicotine exposure. It is also suggested that, extrapolating from knowledge about CC smoking–associated behaviours, ENDS use by adolescents would likely lead to increased reward-seeking behaviours such as the use of other dependence-producing substances. Some studies have suggested that CC smoking in adolescence is associated with impairments in attention and cognition, and studies in rats have shown that adolescent nicotine exposure induces synaptic changes in prefrontal cortical regions responsible for attention, memory, and cognition (*cited ref:* Bergestrom et al., 2008). The potential association of CC smoking with mood disorders in adolescence is also discussed. Animal studies suggest that adolescent nicotine exposure may lead to long-term changes in emotional responses, particularly anxiety and fear (*cited refs:* Slawecki et al., 2003; Smith et al., 2006). In summary, the narrative concludes that 'given the existing evidence from human and animal studies of the detrimental impact of nicotine exposure on adolescent brain development, the use of e-cigarettes by youth should be avoided and actively discouraged. Both preadolescence and adolescence are developmental periods associated with increased vulnerability to nicotine addiction, and exposure to nicotine during these periods may lead to long-lasting changes in behavioural and neuronal plasticity.'

Nicotine exposure from maternal nicotine consumption: prenatal and postnatal health outcomes

16. Nicotine crosses the placenta and has been measured in embryonic tissue as early as seven weeks of gestation. Nicotinic acetylcholine receptors (nAChRs) are widely distributed in fetal brain. Animal studies have shown upregulation of nAChRs and disruption of fetal brain cell replication and differentiation are associated with nicotine exposure (*cited ref:* Slotkin et al., 1998). Prenatal nicotine exposure has also been associated with dysregulation of other neurotransmitter systems. A Cochrane systematic review in 2012 concluded that the effectiveness and safety of NRT during pregnancy is unclear (*cited ref:* Coleman et al., 2012). Some potential effects of nicotine exposure on prenatal development and postnatal outcomes are cited, including sudden infant death syndrome (SIDS), altered development of the corpus callosum, deficits in auditory processing, and alterations in appetite and consumatory behaviours, attention and cognition. Current knowledge regarding each of these aspects is discussed in detail in this section of the publication. It is currently unclear whether, or to what extent, these outcomes are related to nicotine exposure or to other aspects of CC smoking or other exposures or environmental factors during pregnancy.

U.S. National Academy of Sciences (2018)

17. In 2018, the U.S. National Academy of Sciences Committee on the Review of the Health Effects of Electronic Nicotine Delivery Systems published the report 'Public Health Consequences of E-Cigarettes', which reviewed the health effects of E(N)NDS. The full text of this report is available at: http://nationalacademies.org/hmd/Reports/2018/public-health-consequences-of-e-cigarettes.aspx (accessed 17/05/18).

18. The report is structured in three sections: Section I 'E-cigarette Devices, Constituents, and Exposures'; Section II 'Effects of E-Cigarettes on Health'; Section III 'Public Health Implications of E-Cigarettes'. Within Section I, Chapter 4 reviews aspects relating to nicotine. Section II reviews knowledge relating to potential effects of E(N)NDS use on different health endpoints, including dependence and abuse liability (Chapter 8), cardiovascular disease (Chapter 9), cancers (Chapter 10), respiratory diseases (Chapter 11), oral diseases (Chapter 12), developmental and reproductive effects (Chapter 13), injuries and poisonings (Chapter 14). Within each of these chapters, some data relating to specific effects of nicotine exposure are discussed. Content from the NAS report that may be of relevance to assessing the toxicity of nicotine exposure from ENDS aerosols is summarised in the following sections.

Chapter 4 - Nicotine

19. Nicotine concentrations in e-liquids are variable and levels reported on labels are not always accurate, including the presence of nicotine in products labelled as zero-nicotine. In addition, nicotine concentrations are not always labelled.

Concentrations can be reported in various units, for example mg/mL, percent, or amount per cartridge. Nicotine concentrations in e-liquids are often in the range of around 0-20 mg/mL, but levels up to approximately 87 mg/mL have been reported[2]. Nicotine delivery to the aerosol generally increases with device power and is also affected by user puffing characteristics[3].

20. Nicotine is a weak base with a pKa of 8.5 and does not cross membranes rapidly in the protonated state. Thus, the pH of the e-liquid would be expected to have implications for nicotine absorption and pharmacologic effects. This has not been widely studied, however pHs ranging from around 5-10 have been reported, with variable proportions of protonated and unprotonated nicotine present in e-liquids. It is thought likely that flavour additives affect e-liquid pH. Although most e-liquids use PG and/or VG as the solvent base, novel products are being developed (e.g. JUUL^{TM}) that do not contain PG or VG, but contain nicotine base and a weak organic acid (for example, benzoic acid or lactic acid) that forms a nicotine salt for delivery of nicotine.

Pharmacology

21. The report notes that nicotine chemistry, metabolism, disposition kinetics, and pharmacology have been reviewed recently (*cited ref:* England et al., 2017).

22. Nicotine is a tobacco alkaloid that generally comprises around 95% of the alkaloid content of combustible tobacco cigarettes, with most of the nicotine in the (S)-isomer form. Nicotine in e-liquids is released on ENDS use as part of the aerosol particles or as vapour. Particles reach the lungs, where nicotine absorption into the pulmonary venous circulation is rapid, or impact in the mouth and upper airways resulting in slower absorption. In contrast, gas-phase nicotine is expected to be absorbed only in the mouth and upper airways. Nicotine from the pulmonary venous circulation enters the arterial circulation and rapidly crosses the blood-brain barrier, where it binds to nAChRs, resulting in the release of multiple neurotransmitters in the brain: predominantly dopamine, important in drug-induced reward, but also other neurotransmitters including norepinephrine (noradrenaline) (arousal, appetite suppression), acetylcholine (arousal, cognitive enhancement), serotonin (mood modulation, appetite suppression), γ-aminobutyric acid (anxiety and tension reduction), glutamate (learning, memory enhancement), and endorphins (anxiety and tension reduction). Addiction develops as an adaptation to chronic nicotine exposure, with increased AChR binding sites and receptor desensitisation. nAChRs present throughout the autonomic and parasympathetic nervous system allow a wide range of physiologic effects of nicotine. Nicotine intoxication can cause nausea and

[2] In the UK, the Tobacco and Related Products Regulations 2016 states that 'nicotine-containing liquid which is presented for retail sale in an electronic cigarette or refill container must not contain nicotine in excess of 20 milligrams per millilitre' (Part 6, section 36(4))

[3] Data on reported nicotine concentrations in e-liquids and E(N)NDS aerosols are reviewed in the discussion paper, TOX/2018/16

vomiting, and in more severe cases diarrhoea, increased salivation and respiratory secretions, bradycardia, seizures, and respiratory depression.

23. The nAChR includes α, β, γ, and δ subunits, with different combinations of α ($\alpha_{3,4,7}$) and β ($\beta_{2,4}$) subunits thought to mediate the effects of nicotine. $\alpha_4\beta_2$ is the primary receptor that mediates nicotine dependence in humans whereas cardiovascular effects of nicotine are thought to be mediated by the $\alpha_3\beta_4$ nAChR. Nicotine in CC smoke is rapidly absorbed into the pulmonary venous circulation, passing to the systemic arterial circulation and reaching the brain within 15 seconds of a puff. Rapid delivery reinforces the dependence effects of CC smoking. ENDS deliver nicotine in a similar manner to CC, with the potential for high level, rapid delivery. Nicotine delivery to the brain from other forms of NRT is slower, and a considerable amount of orally administered nicotine is swallowed and undergoes first-pass metabolism. This leads to a lesser abuse potential. Nicotine is rapidly absorbed through the skin, thus exposure of the skin to e-liquids can lead to toxicity, which has been observed in an occupational setting in workers handling tobacco plants ('green tobacco sickness'). Absorbed nicotine is extensively distributed to body tissues, with a low affinity for adipose tissue and high affinity for liver, kidney, spleen, lung, and brain. Nicotine accumulates in gastric juice and saliva, in human breast milk, and in fetal serum and amniotic fluids. In CC smokers, blood nicotine levels during daily smoking vary from around 19-50 ng/mL (peak) and 10-37 ng/mL (trough), with an elimination half-life of 2 h. Exposure is thus generally persistent during the day, with levels declining during sleep.

Metabolism

24. Nicotine is metabolised extensively in the liver. Around 70-80% is metabolised to cotinine, via CYP2A6, which shows several polymorphisms, and cotinine is further metabolised to a number of metabolites. Around 4-7% of nicotine is metabolised by FMO3 to nicotine N'-oxide, which is excreted or reduced back to nicotine. Other, minor, metabolic pathways include methylation by N-methyltransferase and glucuronidation by UGT. Around 90% of the systemic dose of nicotine is excreted as metabolites in the urine. Elimination rate varies between humans and across species, affected by factors including genetic polymorphisms, diet, age, gender, pathology, medications, smoking, racial and ethnic differences. Clearance is affected by hepatic blood flow and increases on eating. Menthol inhibits CYP2A6 and nicotine metabolism has been shown to be reduced after smoking mentholated CC in comparison with non-mentholated CC. It is also theoretically likely that other inhibitors of CYP2A6, such as grapefruit juice, will inhibit nicotine metabolism. Individual factors that modulate nicotine metabolism are discussed in detail in the review. Nicotine clearance decreases with age, is higher in women than men, and increases with oral contraceptive use and in pregnancy. Nicotine clearance is reported to be slower in black than white subjects, and slowest in Chinese Americans, which is probably related to prevalences of genetic polymorphisms. Individual nicotine metabolism rate is related to the likelihood of being a smoker and

success rate of quitting. Nicotine metabolism varies across species, including rates, relative amounts of metabolites formed, and variation in metabolising enzymes.

Carcinogenesis

25. The review considers that 'Current evidence does not support the idea that nicotine is a human carcinogen'. One long-term study is noted, the five-year, randomised 'Lung Health Study', which investigated the effects of smoking cessation on lung disease and function, and looked at cancer risk from NRT use (*cited ref:* Murray et al., 2009). No effects on lung, gastrointestinal, or any cancers were observed. A review by the U.S. Surgeon General in 2014 found that most genotoxicity studies were negative (*cited ref:* HHS, 2014). One study showed that mutagenic potential of nicotine in CC smoke did not increase with increasing nicotine concentration (*cited ref:* Chen et al., 2008). One study observed that mice injected intravenously with 3 mg/kg bw nicotine five times per week for 24 months developed sarcomas of the uterus muscle, but no other tumours (*cited ref:* Galitovskiy et al., 2012). The U.S. Surgeon General (*cited ref:* HHS, 2014) concluded that 'there is insufficient data to conclude that nicotine causes or contributes to cancer in humans'. The Surgeon General also concluded that there may be possible oral, oesophageal, or pancreatic cancer risks due to the endogenous formation of TSNA, N'-nitrosonornicotine (NNN) in NRT users and increased risk of these cancers in users of smokeless tobacco products. Mechanistic considerations relevant to the possible effect of nicotine on carcinogenic pathways are cited, including inhibition of apoptosis, stimulation of pathways affecting cell proliferation, stimulation of fibroblast production, and possible promotion of metastasis. These aspects have been reviewed recently (*cited ref:* Grando et al., 2014). A systematic review on the potential carcinogenicity of nicotine (*cited ref:* Haussmann and Fariss, 2016) concluded, based on animal data, that 'limited evidence suggests an association between long-term nicotine exposure and *lack* of a complete carcinogenic effect' and that there is inadequate evidence as to whether or not nicotine modulates carcinogenesis in humans. It is noted that studies of users of smokeless tobacco have in general provided evidence to support a lack of association of nicotine with human cancer risk (although there is evidence of increased risk of cancers related to TSNA exposure) (*cited refs:* Luo et al., 2007; Timberlake et al., 2017). Overall, the narrative considers that although there is biological plausibility that nicotine could act as a tumour promoter, there is no actual evidence of increased risk in NRT and smokeless tobacco product users, and concludes that 'Based on the existing body of evidence, it is reasonable to infer that there is likely no significant increase in risk of cancer from exposure to nicotine delivered by e-cigarettes'.

Cardiovascular effects

26. Cardiovascular effects of nicotine have been reviewed previously (*cited refs:* HHS 2010, 2014; Benowitz and Burbank, 2016) and most data are taken from these reviews. Many of the effects of nicotine on the cardiovascular system are considered to be mediated via nAChR receptors, which are present in endothelial, immune,

neuronal, and muscle cells. Nicotine stimulates the sympathetic nervous system via activation of nAChRs in the peripheral and central nervous system, which can lead to increased heart rate and blood pressure, myocardial contractility, and cutaneous and coronary vasoconstriction. Effects of nicotine on the cardiovascular system may also include: myocardial remodelling, from persistent sympathetic stimulation; arrhythmogenesis, mediated through catecholamine release; thrombogenesis, however, NRT and smokeless tobacco studies do not indicate increased platelet activation on nicotine intake; endothelial dysfunction, mediated by oxidative stress and chronic inflammation (observed in subjects following local nicotine infusion and use of nicotine inhaler); inflammation, although nicotine has shown both anti-inflammatory and pro-inflammatory effects and is not considered to be the main mediator of inflammatory response in CC smokers; angiogenesis, which has been shown to be enhanced by acute exposure to nicotine but impaired on chronic exposure, suggesting that nicotine is not a main player in CC-associated angiogenesis. Some cardiovascular effects associated with CC smoking are described. Smoking is associated with an atherogenic lipid profile, however it is noted that HDL/LDL ratios improve on smoking cessation with NRT use. Smoking CC is also associated with progression of chronic hypertension to accelerated or malignant hypertension, and it is considered likely that nicotine-related vasoconstriction is involved in this progression. Smoking CC is associated with increased insulin resistance and nicotine is considered to be the main factor causing this effect, which has also been observed in long-term users of nicotine gum. Overall the narrative concludes that ‘based on known cardiovascular effects of nicotine…, exposure to nicotine from e-cigarettes likely elevates the risk in people with preexisting cardiovascular disease(s), but the risk in people without cardiovascular disease(s) is uncertain’.

Exposure

27. The final section of Chapter 4 reviews knowledge of nicotine exposure profiles from ENDS use. Twenty-seven clinical studies that evaluated nicotine exposure profiles from ENDS use are reviewed, of which 17 studies looked at inexperienced ENDS users and 12 focussed on experienced users. It is concluded that ‘These studies suggest that e-cigarettes deliver lower levels of nicotine when used by e-cigarette-naïve smokers compared with levels delivered by combustible cigarettes’ (around 1 mg), but also that ‘studies of nicotine delivery and systemic retention in experienced users suggest that e-cigarettes can deliver nicotine in the range of a typical combustible tobacco cigarette, and most of the nicotine is systemically retained under experimental conditions’.

<u>Chapter 8 – Dependence and abuse liability</u>

28. This chapter notes that nicotine is the principal pharmacological agent that causes dependence on combustible cigarettes, and that pulmonary delivery in ‘bolus’ form produces a higher addiction potential relative to other nicotine-delivery devices with slower pharmacokinetics. It is also noted that other, non-nicotine factors,

including environmental and behavioural aspects, are involved in addiction to CC smoking and that while it is plausible that ENDS use may cause dependence, this may also be modulated by factors in addition to the presence of nicotine. Dependence may occur as a synergistic effect of pleasurable sensory experiences (taste, smell, airway sensation, etc.) in combination with nicotine 'bolus' by the pulmonary route. Knowledge from CC smoking may not necessarily be directly transferable to ENDS. Data from studies reported to date is reviewed in detail. It is concluded that: 'There is substantial evidence that e-cigarette use results in symptoms of dependence on e-cigarettes'; 'There is moderate evidence that risk and severity of dependence are lower for e-cigarettes than combustible tobacco cigarettes'; 'There is moderate evidence that variability in e-cigarette product characteristics (nicotine concentration, flavoring, device type, and brand) is an important determinant of risk and severity of e-cigarette dependence'.

Chapter 9 – Cardiovascular disease

29. While the potential implication of nicotine exposure *per se* in cardiovascular disease is addressed in Chapter 4 of the NAS report, Chapter 9 discusses studies that evaluated cardiovascular disease in relation to E(N)NDS aerosol exposures. The chapter is not described further here, as this area is reviewed in the discussion paper on toxicity of E(N)NDS aerosols, TOX/2018/24. The following conclusions were drawn in Chapter 9 of the NAS report:

- 'There is substantial evidence that heart rate increases shortly after nicotine intake from e-cigarettes'.
- 'There is moderate evidence that diastolic blood pressure increases shortly after nicotine intake from e-cigarettes'.
- 'There is limited evidence that e-cigarette use is associated with a short-term increase in systolic blood pressure, changes in biomarkers of oxidative stress, increased endothelial dysfunction and arterial stiffness, and autonomic control'.
- 'There is insufficient evidence that e-cigarette use is associated with long-term changes in heart rate, blood pressure, and cardiac geometry and function'.

Chapter 10 - Cancers

30. While the potential carcinogenicity of nicotine is addressed in Chapter 4 of the NAS report, Chapter 10 addresses data on cancer in relation to E(N)NDS aerosol exposures. A section of this chapter addresses studies of major E(N)NDS components on cancer outcomes, including discussion of nicotine.

31. It is noted that evaluation of the potential carcinogenicity of nicotine via epidemiological studies is complicated by the fact that users of the 'purest' form of nicotine, NRT, are generally quitting CC smokers. One study is cited as useful, namely the Lung Health Study (as mentioned in paragraph 25), in which the participants' use of NRT (nicotine gum) and CC was documented over 5 years, and 3320 subjects were followed-up for a further 7.5 years (*cited ref:* Murray et al., 2009).

No increased risk of lung, gastrointestinal, or all cancers was observed. However, it is noted that 7.5 years of follow-up is not overly informative in terms of cancer as the endpoint (apart from effects on later stages of carcinogenesis), and that the sample size was small in terms of statistical power. The narrative notes that several studies have evaluated long-term exposure to nicotine in animal models, and these data have been reviewed recently (*cited ref:* Haussmann and Fariss, 2016). A study in which adult female rats were constantly exposed to nicotine by inhalation (501 ± 151 mg/m^3) for 2 years showed that more animals developed tumours in the exposed (21/59) than control (6/25) group, although these tumours were common to the strain of rat. Pituitary gland adenomas were observed only in treated animals (*cited ref:* Waldum et al., 1996). Data from this study are summarised in Table 10-4 of the NAS report (page 10-16). Another study evaluated lifetime exposure to nicotine in drinking water (0.5 or 0.7 mg/mL, equivalent to approximately 150 mg/kg bw/day) in male and female Swiss mice. No effects of toxicity were observed and there was no increase in tumour incidence in the treated animals (*cited ref:* Toth et al., 1982). This was described as a generally well designed study, but it was noted that the nicotine dose evaluated may not have been sufficiently high. One study indicated that nicotine (0.15 mg/animal/day[4] for 46 weeks) did not increase lung tumour multiplicity nor enhance the development of NNK-induced lung tumours in A/J mice (*cited ref:* Murphy et al., 2011). A study in rats investigated potential effects of oral nicotine exposure (nicotine hydrogen tartrate; 52 ppm (rats), 514 ppm (mice), for 4 weeks in drinking water) on early-stage bladder carcinogenesis. Histological evaluation of the urothelial lining showed hyperplasia in 70% and 40% of treated rats and mice, respectively, but not in control groups (*cited ref:* Dodmane et al., 2014).

32. In summary to Chapter 10, the NAS report concludes that 'The sparseness of the current evidence and the low quality of the human evidence on this topic preclude making any evidence-based conclusions about the potential association between e-cigarette use and risk of cancer in human populations'. No concluding comments are made relating specifically to nicotine.

Chapter 11 – Respiratory diseases

33. This chapter addresses the potential effects of E(N)NDS aerosol exposures on respiratory diseases, including some discussion of aspects relating specifically to nicotine content.

34. The introductory section notes that nicotine-containing ENDS aerosols have the potential to adversely impact several host-defence mechanisms in the lungs, and discusses mechanisms via which such effects may be mediated, including possible impairment of cystic fibrosis transmembrane conductance regulator (CFTR) activity in the airways via downregulation of α7nAChR activity, leading to reduced mucociliary clearance (MCC).

[4] The NAS report does not mention the route of exposure of nicotine in this study

35. Short-term clinical studies in humans and studies in animals are reviewed, which have evaluated effects of E(N)NDS aerosol exposures, with or without nicotine, on respiratory parameters. (These data are reviewed in the discussion paper on toxicity of E(N)NDS aerosols, TOX/2018/24). Short-term exposure of human subjects to nicotine in ENDS has been associated with adverse effects on lung defence mechanisms, including suppressed cough and MCC (*cited refs:* Dicpinigaitis et al., 2016; Kumral et al., 2016). Some animal studies have indicated possible adverse effects of nicotine in ENDS aerosols on the respiratory system, including increased airway hyper-reactivity and impairment of MCC in mice (*cited refs:* Garcia-Arcos et al., 2016; Laube et al., 2017). One study indicated that neonatal exposure of mice to nicotine-containing ENDS aerosol led to diminished alveolar proliferation and impaired lung growth (*cited ref:* McGrath-Morrow et al., 2015). However, it was noted that interpretation of the animal data set is limited by the different methodologies used and by lack of standardisation, including lack of evaluation of biomarkers of systemic nicotine absorption. A section on vulnerable populations describes the potential for nicotine exposure from ENDS use to increase the rate of respiratory symptoms in cystic fibrosis (CF) carriers if nicotine causes dysregulation of CFTR in the airways, and to have long-lasting negative effects on lung function in preterm infants.

Chapter 12 – Oral diseases

36. This chapter notes that a small number of *in vitro* studies have indicated a detrimental effect of nicotine, as well as of other constituents of E(N)NDS aerosols (e.g. flavourings), on cell viability of epithelial and fibroblast cells in culture, indicating that E(N)NDS aerosols may be harmful to oral cavity cells.

Chapter 13 – Developmental and reproductive effects

37. Smoking CC is associated with adverse pregnancy outcomes including placental abruption, ectopic pregnancy, pre-term birth, fetal growth restriction, still-birth, infant mortality, SIDS, and orofacial cleft. The agents within CC smoke that are responsible for these effects are not known, but nicotine is a likely contributor. Some, albeit limited, data are available from NRT use during pregnancy. One study indicated improved birth weight in users of NRT rather than CC during pregnancy, with equivalent nicotine intakes, implicating constituents other than nicotine in CC (*cited ref:* Wisborg et al., 2000), but another study did not observe this effect (*cited ref:* Coleman et al., 2012). No data were available on the use of ENDS during pregnancy in humans. Some studies on adverse reproductive outcomes of CC use in pregnancy are reviewed in this chapter. The narrative notes that nicotine crosses the maternal placental barrier, that nAChRs are present in fetal brain and lungs, and that fetal and infant nicotine metabolism is slower than in adults. One study that evaluated congenital abnormalities in children of mothers who smoked CC or used NRT during pregnancy did not observe differences between the two groups except for an increase in respiratory anomalies in children of NRT users (*cited ref:* Dhalwani et al., 2015). A database of animal studies is also reviewed. Nicotine has been

shown to adversely affect fetal airway development and lung histology. Rhesus monkeys implanted with nicotine pumps during pregnancy produced offspring with reduced total body weight and alveolar hyperplasia with upregulation of α_7nAChRs in airway cartilage and vessels of fetal lungs (*cited ref:* Sekhon et al., 1999). Exposure to nicotine during gestation and the early postnatal period was shown to disrupt vascularisation and alter lung development in rodents, without effects on lung function (*cited ref:* Petre et al., 2011). Studies have shown that prenatal nicotine exposure stimulates lung branching through α_7nAChRs in mouse lung explants, and offspring of mice with prenatal nicotine exposure had decreased forced expiratory flow and decreased airway diameters (*cited refs:* Wongtrakool et al., 2007, 2012). Whole-body exposure of mice during gestation and early postnatal period to nicotine-containing ENDS aerosols was associated with behavioural alterations in adult offspring (*cited ref:* Smith et al., 2015), while neonatal ENDS aerosol exposure in another study was associated with impaired alveolar growth and decreased lung cell proliferation (*cited ref:* McGrath-Morrow et al., 2015)[5].

38. The NAS narrative notes that although animal studies have demonstrated an adverse effect of nicotine on lung development and postnatal lung function and behaviour, dose-response data are not available. In addition, the exposure routes used in these studies (including pumps, injections, whole-body exposures) may not be applicable to human exposures. In the studies of E(N)NDS aerosol exposures, it is not always possible to determine the effects of nicotine separately from other aerosol parameters, such as particle size and other components such as flavourings.

Chapter 14 – Injuries and poisonings

39. This chapter reports that ingestion of nicotine-containing e-liquids can result in serious toxicity. Case reports are described in which poisonings have occurred via oral or dermal routes. Effects have included vomiting, lactic acidosis, and in some cases, death. Many cases relate to ingestion by young children. The report concludes that ‘There is conclusive evidence that intentional or accidental exposure to e-liquids (from drinking, eye contact, or dermal contact) can result in adverse health effects including but not limited to seizures, anoxic brain injury, vomiting, and lactic acidosis’, and ‘There is conclusive evidence that intentionally or unintentionally drinking or injecting e-liquids can be fatal’.

Summary

40. Information relevant to the toxicity of nicotine by exposure from ENDS is summarised, taken from three recent reviews (RCP, 2016; USHHS, 2016; NAS, 2018).

41. Nicotine in e-liquids is released on ENDS use on the aerosol particles or as vapour. The amount of aerosolized nicotine available for inhalation from ENDS is modulated by factors including nicotine concentration in the e-liquid, power (voltage

[5] Studies of E(N)NDS aerosol exposure are reviewed in the discussion paper, TOX/2018/24

and resistance) of the ENDS device, and user behaviour (puffing topography). Particles reach the lungs, where nicotine absorption into the pulmonary venous circulation is rapid, or impact in the mouth and upper airways with slower absorption. Gas-phase nicotine is expected to be absorbed in the mouth and upper airways. Nicotine from the pulmonary venous circulation enters the arterial circulation and rapidly crosses the blood-brain barrier, where it binds to nAChRs, resulting in the release of multiple neurotransmitters in the brain. Addiction develops as an adaptation to chronic nicotine exposure, with increased AChR binding sites and receptor desensitisation. Rapid delivery reinforces the dependence effects of CC smoking. ENDS deliver nicotine in a similar manner to CC, with the potential for high level, rapid delivery and ENDS users are reported to develop the capability to extract more nicotine from the devices as they become more experienced with use. Nicotine delivery from other forms of NRT is slower, and orally delivered nicotine undergoes first-pass metabolism.

42. Nicotine is metabolised extensively in the liver. Around 70-80% is metabolised to cotinine, via CYP2A6, which shows several polymorphisms, and cotinine is further metabolised to a number of metabolites. Around 90% of the systemic dose of nicotine is excreted as metabolites in the urine. Elimination rate varies between humans and across species, affected by factors including genetic polymorphisms, diet, age, gender, pathology, medications, smoking, racial and ethnic differences. Clearance is affected by hepatic blood flow and increases on eating. Individual nicotine metabolism rate is related to the likelihood of being a smoker and success rate of quitting. Nicotine metabolism varies across species, including rates, relative amounts of metabolites formed, and variation in metabolising enzymes.

43. Nicotine intoxication can cause nausea and vomiting, and in more severe cases diarrhoea, increased salivation and respiratory secretions, bradycardia, seizures, and respiratory depression. Case reports have described poisonings from e-liquids containing nicotine. Effects have included vomiting, lactic acidosis, and in some cases, death (by ingestion or injection). Many cases relate to ingestion by young children. The LD_{50} for nicotine in humans is cited recently as 6.5-13 mg/kg bw.

44. Nicotine has acute effects on the cardiovascular system, including increased heart rate and blood pressure. In the longer term, smoking CC is associated with adverse cardiovascular effects, for some of which nicotine exposure may be implicated (including progression of chronic hypertension to accelerated or malignant hypertension, and increased insulin resistance). NAS (2018) concluded that ‘exposure to nicotine from e-cigarettes likely elevates the risk in people with preexisting cardiovascular disease(s), but the risk in people without cardiovascular disease(s) is uncertain’. Data on longer term effects on the cardiovascular system of nicotine exposure from ENDS is not currently available.

45. Nicotine exposure from ENDS has been shown to have adverse effects in the short term on the respiratory system, including suppression of cough and mucociliary

clearance in humans, and increased airways hyper-reactivity and impaired mucociliary clearance in animals.

46. Animal studies have indicated that nicotine exposure can have negative effects on fetal and adolescent brain development, and one study showed behavioural effects in adulthood on offspring of mice whole-body exposed to nicotine-containing ENDS aerosol during gestation and early postnatal development. Animal studies have also shown that nicotine exposure can adversely affect fetal airway development and lung histology, including one study in which neonatal mice were exposed to nicotine in ENDS aerosol.

47. Nicotine is not considered to be a human carcinogen and has generally been found to be negative in genotoxicity studies.

Questions for the Committee

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

i. Do Members wish for any of the data highlighted in this preliminary overview of the potential toxicity of nicotine exposure from ENDS aerosols to be reviewed in more detail?

ii. Are there any 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

AChRs	Acetylcholine receptors
BP	Blood pressure
CC	Conventional cigarette
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CRP	C-reactive protein
CYP	Cytochrome P450 enyzme
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
FMO	Flavin-containing monooxygenase
GABA	γ-aminobutyric acid
HDL	High density lipoprotein
LD_{50}	Lethal dose 50
LDL	Low-density lipoprotein
MOA	Monoamine oxidase
MCC	Mucociliary clearance
nAChR	Nicotinic acetylcholine receptor
NAS	U.S. National Academy of Sciences
NMR	Nicotine metabolite ratio
NNK	Nicotine-derived nitrosamine ketone
NNN	N-nitrosonornicotine
NRT	Nicotine replacement therapy
PAH	Polycyclic aromatic hydrocarbon
PG	Propylene glycol
SIDS	Sudden infant death syndrome
TSNA	Tobacco-specific nitrosamine
UGT	Uridine diphosphate glucuronyltransferase
VG	Vegetable glycerin(e) (glycerol)
VOC	Volatile organic compound

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