CC/2019/17

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes) – update of available data on carcinogenicity.

Background

1. The COT is reviewing the potential toxicity of electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (collectively abbreviated to E(N)NDS). As part of this review, new literature of relevance to this topic is kept under surveillance, and a recent literature update identified publications of relevance to the potential carcinogenicity of E(N)NDS. COC previously reviewed literature relating to this topic at the July 2018 meeting (CC/2018/01). This current paper presents findings from an updated literature search, for the Committee to consider whether this provides any new information on potential carcinogenicity of E(N)NDS to be highlighted to the COT.

Introduction

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

3. The principal components of most e-liquids are the solvents, propylene glycol (PG) and glycerol. Other common additives are water, nicotine, and flavourants, as well as sweeteners and flavour enhancers. Some studies have reported the presence of contaminants and impurities in e-liquids, often at low or trace levels, including ethylene glycol, nicotine-related contaminants (minor tobacco alkaloids and tobacco-specific nitrosamines), diethyl phthalate and diethylhexyl phthalate, and ethanol. Active compounds such as a weight-loss drugs and a synthetic cannabinoids have been detected in some individual e-liquid samples. Aerosol is produced by heating the e-liquid within the device. The aerosol comprises two main parts – a particulate (droplet) phase and a gas (vapour) phase. Thermal decomposition of e-liquids during aerosol production may lead to the production of degradation products, for example carbonyl compounds such as formaldehyde, acetaldehyde, and acrolein, although there is some debate about whether this would actually occur during 'real-life' use of E(N)NDS by users or is an artefact of experimental machine-puffing protocols.

COC conclusion in July 2018

4. At the July 2018 meeting, the COC considered paper CC/2018/01, and concluded:

> "The COC concluded that relative risk of E(N)NDS compared to conventional cigarettes appeared to be lower, but there was still some risk associated with the chemicals and particles in the emissions from E(N)NDS. This risk should be emphasised to new users. In addition. Members concluded that the possibility of bystander effects should also be considered." (CC/MIN/2018/01)

5. The paper and full minutes of July 2018 discussion are attached at Annex A

Literature search

6. An updated literature search was conducted for the period June 2018 to present to identify publications of relevance to carcinogenicity of E(N)NDS. Details of the search string are provided at Annex B. Excluding review articles, two publications were identified as needing consideration by COC. The publication of Tang, et al. (2019) describes a study in which tumourigenic effects of exposure to E(N)NDS aerosol were evaluated in mice. A study reported by Zahedi, et al. (2018) investigated potential effects of e-liquids and aerosols on epithelial to mesenchymal transition in lung cancer cells *in vitro*. These are described below.

Tang et al. (2019)

7. This study is a follow-on from a study reported by Lee, et al. (2018), which was reviewed by COT (TOX/2018/24) and COM (MUT/2018/08) in 2018. Summaries of the Lee et al. (2018) paper, taken from the COT and COM discussion papers, are reproduced at Annex C.

8. In the study reported by Tang, et al. (2019), 6-8 week-old male FVB/N mice were whole-body exposed over a 54-week period to either filtered air (FA) (n=20), a 1:1 PG/glycerol mix ('vehicle', Veh) (n=20), or vehicle containing 36 mg/mL nicotine ('e-cig smoke', ECS) (n=45)[1]. Test exposures were given for 4 hours/day, 5 days/week. The narrative of the report describes that mice in the FA group "remained housed in the animal room, exposed to the ambient filtered air", while for Veh and ECS test groups, exposures were administered in an exposure chamber (referring to the protocol previously described in Lee et al. 2018[2]). In contrast, information in the supplementary files of Tang, et al. (2019) notes exposure chamber concentrations of 130 mg/m^3 particulate matter for all three groups. Exposure chamber concentrations for nicotine of below the limit of detection (LOD) (< 0.000394 mg/m^3) for the FA group, 0.00516 mg/m^3 for the Veh group, and 0.196 mg/m^3 for the ECS group were also reported. Over the 54-week period, a total of 6 mice were found dead (2 FA, 1 Veh, 3 ECS) and 3 were euthanized (1 Veh due to a paralysed leg, 2 ECS due to inactiveness). No lung tumours were observed in these mice, while one of the ECS mice had a large intestinal polyp.

9. At the end of the 54-week period, mice were euthanized and lungs, heart, liver, kidneys, intestine, pancreas, brain, spleen, and bladder were examined for visible tumours. Organs were fixed and sections were examined by H&E staining. In addition, bladder sections were examined for the cell proliferation markers, MCM-2 and PCNA, and the basal cell marker, KRT5.

10. Tumour-like growths were observed in skin, abdominal cavity, intestine and lungs. Data are summarised in Table 1[3].

[1] Details of the source or purity of the reagents used were not given.

[2] In Lee et al. (2018), mice were exposed in a 1 m^3 exposure chamber, into which aerosol generated from a tank-system E(N)NDS device was mixed with filtered air and pumped into the chamber.

[3] The overall incidence of spontaneous tumours in male FVB/N breeder mice has been reported as 45%; most of the tumours found in mice over 600 days (86 weeks) and almost all of the tumours were alveolar-bronchial tumours of the lungs (Huang 2008). Another study that evaluated incidence of neoplastic disease in FVB/N mice at 14 and 24 months found that the incidence of tumour-bearing male mice was 13% at 14 months and 55% at 24 months (Mahler et al. 1996). Based on the 14-month data, the background incidence of tumours in the 14-month-old mice in the study of Tang et al. (2019) could be calculated as 2.34 per 18 mice or 5.2 per 40 mice.

Table 1. Numbers of mice per treatment group with tumours in different organs.

	Number of mice with tumour		
Treatment group (number of mice in group to end of study)	FA (n=18)	Veh (n=18)	ECS (n=40)
Lung	1	0	9*
Bladder	0	0	0
Intestine	1	1	1
Abdominal cavity	0	1	2
Skin	0	1	1
Liver, heart, brain, spleen, kidney	0	0	0

* One of the 9 tumour samples was lost before H&E staining could be performed.

11. Lung tumours were present in 9/40 (22.4%) mice exposed to ECS and 1/18 (5.6%) mice exposed to FA. Of the nine ECS-exposed mice, eight had a single adenocarcinoma and one had multiple ipsilateral lung adenocarcinomas. The FA-exposed mouse had a single adenocarcinoma.

12. Adenomatous polyps with high-grade dysplasia were found in the intestine in 1 FA, 1 Veh, and 1 ECS mouse. A cystic salivary benign tumour was found in 1 Veh and 1 ECS mouse. Tumour-like growth of skin in 1 Veh mouse was necrotic tissue that could not be further characterised. Skin tumour-like occurrence in 1 ECS mouse contained muscle and bone and was reported as negative for tumour. Simple or nodular hyperplastic changes in the bladder urothelium were observed in 0/17 FA, 1/16 Veh and 23/40 ECS mice (1 sample from FA-exposed mice and 2 samples from Veh-exposed mice were inadvertently destroyed).

13. *P* values, relative risks, and 95% confidence intervals (95% CI) were calculated for lung tumours according to ‘mice with tumour’ and ‘mice without tumour’. Analysis by treatment group showed a significant increase in incidence in ECS vs. Veh, ECS vs. FA+Veh, and ECS vs. FA vs. Veh, but not in ECS vs. FA. Results are summarised in Table 2.

Table 2. Statistical analysis of lung tumour incidence by treatment group.

Comparison	Calculated based on 9 or 8 mice with tumours in the ECS group*	*P* value	Relative risk	95% CI
ECS vs. FA	9	0.1498	4.05	0.7733 - 24.14
	8	0.2467		
ECS vs. Veh	9	0.0454	Infinity	1.22 - infinity
	8	0.0463		
ECS vs. (FA + Veh)	9	0.0154	8.1	1.445 – 48.43
	8	0.0295		
ECS vs. FA vs. Veh	9	0.0352	Unpredictable	Unpredictable
	8	0.054		

* Main results were presented for 9 mice with lung tumours in the ECS group (i.e. including 1 mouse for which the tumour was not analysed by H&E staining). *P* values were also provided for analyses excluding this mouse.

14. The authors considered that these results indicated that nicotine exposure, as administered by the protocol of this study, induced lung adenocarcinoma in mice. They commented that current opinion generally considers that nicotine is not carcinogenic, although good quality studies are generally not available. Only one inhalation exposure study has been noted, in which rats exposed to stream-air vaporised nicotine for 2 years showed no increased tumour formation, including lung tumours (Waldum, et al. 1996), but this study had several shortcomings. Tang and colleagues suggested that a hypothesis for the discrepancy between their findings and those of Waldum et al. might be a difference in inhaled particle size, whereby smaller particles produced from E(N)NDS could allow deep penetration of nicotine into the lung, rather than deposition in the upper aerodigestive tissues. Subsequently, nicotine nitrosation products formed in the lung may cause DNA damage, with the formation of γ-OH-PdG and O^6-methyl-dG adducts, as observed in their previous study (Lee et al. 2018).

15. Overall, Tang et al. concluded that exposure of mice to E(N)NDS aerosol containing nicotine induced lung cancer and bladder urothelial hyperplasia. They commented that "These findings, combined with our previous findings [Lee et al. 2018] that ECS[4] induces γ-OH-PdG and O^6-methyl-dG adducts in the lungs and bladder urothelium and inhibits DNA repair in lung tissues in mice, and that nicotine and NNK induce the same types of DNA adducts and DNA repair inhibition effect

[4] i.e. E(N)NDS aerosol containing nicotine.

and sensitize mutational and tumorigenic cell transformation susceptibility in the human lung epithelial and urothelial cells, indicate that ECS, as well as nicotine and NNK, is a lung carcinogen and a potential bladder carcinogen in mice."

Zahedi et al. (2018)

16. This study by Zahedi, et al. (2018) evaluated the effect of exposure to e-liquid or aerosol produced from E(N)NDS on epithelial to mesenchymal transition (EMT) in A549 human lung adenocarcinoma basal epithelial cells.

17. Two commercial E(N)NDS products purchased in California were tested. The products were labelled to contain PG, glycerol, and 48 mg/mL nicotine. The presence of flavourings was not listed, but the products were named as tobacco and menthol flavours, respectively. No analysis of the e-liquids for chemical content was reported. Aerosols were produced by machine-puffing e-liquid diluted to 1% (no further detail provided) and collected into A549 culture medium (6 puffs/mL) in a 250 mL flask on dry ice. The medium was then frozen until use.

18. A549 cells were cultured and maintained in either control medium or medium containing dilutions of e-liquid or aerosol for 3 – 8 days. Further details of product dilutions were not specified.

19. Exposure of cells to medium containing e-liquid or aerosol was reported to lead to acquisition of a fibroblast-like morphology, loss of cell-to-cell junctions, internalization of E-cadherin, increased motility, and upregulation of EMT markers. This was concurrent with plasma membrane to nuclear translocation of active β-catenin. Authors concluded that EMT was induced in A549 cells by both the menthol and tobacco flavoured e-liquids and aerosols from the product used in this study, and that the factor causing the effect was thus present in the e-liquid, but not produced by aerosolisation.

20. Authors commented that EMT is an initial step in tumour metastasis and thus such an effect associated with E(N)NDS exposure may have implications for former or heavy cigarette smokers who are at increased risk for lung cancer or may already have a lung tumour.

Questions for the Committee

21. Members are asked to consider this paper and in particular:

i. Do any of the data presented from this updated literature search provide important new information on potential carcinogenicity of E(N)NDS?

ii. Are there any particular aspects of this paper that should be highlighted to the COT for its consideration?

NCET at WRc/IEH-C under contract supporting the PHE COC Secretariat
October 2019

Abbreviations/Glossary

CC	Conventional cigarette
ECS	‘e-cig smoke’
EMT	Epithelial to mesenchymal transition
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
FA	Filtered air
LOD	Limit of detection
PG	Propylene glycol

References

Huang, P., et al. 2008 Histopathologic findings and establishment of novel tumor lines from spontaneous tumors in FVB/N mice. Comp Med 58(3):253-63.

Lee, H. W., et al. 2018 E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. Proc Natl Acad Sci U S A 115(7):E1560-e1569.

Mahler, J. F., et al. 1996 Spontaneous lesions in aging FVB/N mice. Toxicol Pathol 24(6):710-6.

Tang, M. S., et al. 2019 Electronic-cigarette smoke induces lung adenocarcinoma and bladder urothelial hyperplasia in mice. Proc Natl Acad Sci U S A.

Waldum, H. L., et al. 1996 Long-term effects of inhaled nicotine. Life Sci 58(16):1339-46.

Zahedi, A., et al. 2018 Epithelial-to-mesenchymal transition of A549 lung cancer cells exposed to electronic cigarettes. Lung Cancer 122:224-233.

CC/2019/17 - Annex A

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes) – update of available data on carcinogenicity.

Previous paper (CC/2018/01) and minutes of its discussion

NCET at WRc/IEH-C under contract supporting the PHE COC Secretariat
October 2019

COC/2018/01

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (EN(N)DS – e-cigarettes) – overview of available data on carcinogenicity.

Background

1. The COT is currently reviewing the possible human health effects of electronic nicotine (and non-nicotine) delivery systems (EN(N)DS, 'e-cigarettes'). A paper (TOX/2018/16) was presented to the COT in which literature searches and full list of publications retrieved for genotoxicity and carcinogenicity of E(N)NDS were presented. After follow-up analysis of the abstracts obtained, it was agreed that the COC and COM should consider the available papers on carcinogenicity and genotoxicity respectively. The aim is for COC (and COM) to assess absolute risks from E(N)NDS and relative risk compared to conventional cigarettes, and if data are available to heated tobacco products.

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 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[1].

[1] see, http://ecigclopedia.com/the-4-generations-of-electronic-cigarettes/ (accessed 04/06/18)

3. A total of 178 references were retrieved from the initial searches and screened for relevance to COC and COM. Of these, 4 papers were identified as needing consideration by COC. Details of the search string are provided in Annex 1. In addition, a recent National Academies of Sciences, Engineering and Medicine (NAS) Report has been published which comprises a systematic review of current science to inform the understanding of public health risks and benefits of e-cigarettes. Chapter 10 of this report outlines the evidence on cancer and is attached at Annex 2. These papers are discussed, and a summary of the conclusions of the NAS report regarding carcinogenicity given, in the following sections.

EN(N)DS literature relating to carcinogenesis

4. Canistro et al. (2017) undertook an assessment of the potential harmful toxicological effects of e-cigarettes that may translate to enhanced risk of cancer in users. The authors used a rat lung model to assess the mutagenic and cancer-initiating potential of the aerosol of the E(N)NDS liquid 'Essential cloud, red fruit flavour'. *Only findings for the cancer-initiating events are discussed in detail here.* The liquid contains (per 100g of product): propylene glycol (PG), vegetable glycerine (VG), deionised water, flavours ("red fruits"), and nicotine (18 mg/mL). The liquid was delivered using a commercial e-cigarette (brand not stated) comprised of a 2.5 mL liquid tank in Pyrex glass and dual coil, using a voltage of 5.5V and wattage of around 15 W.

5. Male Sprague Dawley rats (8 weeks of age) were exposed by whole body inhalation to the E(N)NDS aerosol containing 18 mg nicotine (equivalent to 1 mL of liquid). The liquid was delivered in 11 cycles comprising 17 sec puff (6 sec on, 5 sec off, 6 sec on) and 20 min stop. Following each cycle animals were transferred to a clean chamber for delivery of the next cycle. Animals were treated to 11 cycles per day for 5 days per week for 4 weeks[2] after which animals were killed and lung microsomes made.

6. The major components of the volatile organic compound (VOC) profile emitted from heating the 'red fruit' liquid were PG, nicotine and VG. Minor components included 1,2-propanediamine, methyl propionate (flavour compound), indole, propanoic acid 1-methylpropyl ester, acetol, 1-methoxy-2-propyl acetate, 3-hexen-1-ol (flavour compound), diacetyl (flavour compound) and acrolein. These findings are in agreement with other published literature, however no formaldehyde was detected which the authors suggest is due to the type of VOC analysis undertaken by them. VOC composition was measured throughout the duration of exposure and within different chambers, and no statistically different differences were found.

7. Modulation of several carcinogen-metabolising enzymes involving cytochrome P450 (CYP450) was observed in the microsomal lung fractions of rats exposed to VOCs from e-cigarettes using several specific probes. A significant increase was

[2] Note that a small number of rats (n=5) received a single i.p. dose of mitomycin C (1 mg/kg bw) as a positive control for the micronucleus test.

observed in several CYP-linked monooxygenases when compared to the control group (non-exposed):

a. CYP1A1/2 which is linked with the activation of pre-carcinogens including polychlorinated biphenyls, aromatic amines, dioxins and PAHs ($p<0.01$):

b. CYP2B1/2 which is linked with the activation of olefins and halogenated hydrocarbons ($p<0.01$);

c. CYP2C11 which is linked to the activation of nitrosamines and mycotoxins ($p < 0.05$);

d. CYP3A which is linked to the activation of hexamethyl phosphoramide and nitrosamines ($p< 0.01$).

8. CYP induction is known to result in enhanced production of reactive oxygen species (ROS), which plays a key role in the cancer occurrence via a co-carcinogenesis mechanism. This was assessed by the authors using an electron paramagnetic resonance (EPR)-radical probe to evaluate the ROS content of the rat lungs. Exposure to e-cigarette aerosol was associated with a significant increase ($p <0.01$) in ROS/oxidative stress in the lungs of exposed rats compared with controls. Simultaneous measurements of the antioxidant enzymes catalase, DT-diaphorase and superoxide dismutase showed these to be significantly reduced ($p <0.01$) following exposure. Systemic antioxidant capacity (measured as ferric reducing antioxidant power (FRAP)) was also reduced in the lungs ($p <0.05$) of exposed rats.

9. From a mutagenic perspective, DNA damage (measured as increased tail length in the Comet assay) was observed in leucocytes, an increase in the percentage of immature micronucleated reticulocytes over normal reticulocyte indicative of chromosome fragmentation (possibly to the mitotic spindle or centromeres) and a positive Ames test in the urine. These aspects of this paper have been presented to the COM in more detail (MUT/2018/08).

10. The authors note that their findings relate to E(N)NDS vapour as a whole and not to individual components. In addition, the vaping conditions used were not reflective of human use but were used only as a preliminary investigation of pre-carcinogenic events.

11. The authors considered that if these findings were extrapolated to humans this would predispose an individual to an enhanced [lung] cancer risk. No quantitation of risk was provided by the authors to support this statement and, as such, these findings cannot be utilised for risk assessment purposes.

12. Fuller et al. (2018) carried out an assessment of the presence of known bladder carcinogenic amines and polycyclic aromatic hydrocarbon (PAH) metabolites in the urine of E(N)NDS users to better understand the risk profile associated with

their use. Urine samples were collected from non-smoking E(N)NDS users (n=13; average age 30.1 ± 7.7 years) and non-smoking, non- E(N)NDS using-controls (n=10; average age 39.4 ± 13.5 years); no information is given by the authors concerning the timing or duration of urine collection. All subjects were former smokers (average duration of 19.9 ± 11.9 years) but had not used conventional cigarettes (CC) for > 6 months prior to sampling. A variety of E(N)NDS devices were used by the exposed group and the frequency of use was >28 times a week for the majority (84.6% of individuals). Samples were analysed by LC-MS for the target compounds benz(a)anthracene, benzo(a)pyrene, 1-hydroxypyrene, o-toluidine and 2-naphthylamine.

13. The E(N)NDS users were found to have statistically significantly higher levels of the known carcinogens o-toluidine ($p = 0.0013$) and 2-napthylamine ($p = 0.014$) when compared to control subjects. PAHs were not detected, however, as the authors do not give details of the level of quantitation of the PAHs using their methodology, it is not possible to interpret these findings here.

14. As all subjects, including the controls, has been previous CC smokers, the authors used a Pearson correlation analysis to compare time since cessation of smoking and carcinogenic metabolite concentration. No correlation was found for either metabolite, with Pearson coefficients of 0.51 and 0.07 for 2-napththylamine and for o-toluidine respectively.

15. The authors conclude that the presence of known bladder carcinogens in the urine of users may suggest the E(N)NDS devices are not risk free from a bladder cancer perspective. However, there is no attempt to qualify the degree of risk in comparison to CC smokers.

16. The excess lifetime cancer risk (ELCR) associated specifically with the inhalation of particles within EN(N)DS aerosol in humans has been evaluated through generation of data on particle concentration and size range (to include sub-micron and super-micron particles) in combination with published information on particle mass, heavy metal content and tobacco-specific nitrosamines (Scungio et al., 2018). The authors measured particle-specific data for two scenarios under the same smoking pattern, i.e. puffs per EN(N)DS and puff duration:

a. exposure to mainstream aerosol (collected directly from the EN(N)DS mouthpiece); and

b. exposure to second hand aerosol (collected in a 40 m^3 naturally ventilated room with an air exchange rate of 0.2 h^{-1}, occupied by users of EN(N)DS vaping under the stated patterns).

17. Particle number and surface area concentration of generated aerosols were determined using a Condensation Particle Counter, with detection at levels to 4 nm diameter. Size distribution and total concentration were measured using a Mobility Particle Sizer spectrophotometer; for the direct exposure scenario, temperatures of

37^{o}C and 300^{o}C were selected to simulate the respiratory system conditions and to evaluate volatility respectively.

18. Using data from available literature, the authors determined that a number of IARC Group 1 carcinogenic compounds have been measured in mainstream and second-hand aerosols from EN(N)DS. These include the heavy metals, cadmium and nickel, arsenic and the nicotine specific nitrosamines nicotine-derived nitrosamine ketone (NNK) and *N*-nitrosonornicotine (NNN). The ELCR for both scenarios was estimated using a Monte Carlo method that was applied by varying the input data between the available measured values, i.e. concentration of hazardous compound, particle number and size distribution[3], surface area, PM_{10}, vaping patterns and e-cigarette consumption.

19. In mainstream EN(N)DS aerosol, the authors reported higher average particle numbers (2.34 ± 0.5 ×10^{8} and 2.23 ± 0.8 and part. cm^{-3} with and without nicotine, respectively at 37^{o}C) when compared with mainstream smoke of CC (data for comparison taken from published studies). At the higher temperature (300^{o}C) particle numbers were lower, both with and without nicotine (7.02 ± 0.8 and 6.23 ± 0.5 x 10^{7} part.cm^{-3} respectively), than in mainstream EN(N)DS aerosols at 37^{o}C (no comparison given by the authors to mainstream smoke of CC).

20. In second-hand EN(N)DS aerosol, particle numbers were considerably lower than in mainstream EN(N)DS aerosol for all combinations of parameters, i.e. at 37^{o}C with and without nicotine (9.08 ± 0.2 and 6.30 ± 1.3 x 10^{3} part.cm^{-3} respectively) and at 300^{o}C with and without nicotine (8.92 ± 0.2 and 5.97 ± 1.3 x 10^{3} part.cm^{-3} respectively.

21. With regards to surface area, the authors reported that EN(N)DS aerosol contained particles of lower surface area (5.22 ± 1.5 and 6.99 ± 0.8 x 10^{11} nm^{2} cm^{-3}, with and without nicotine respectively) at 37^{o}C when compared with mainstream smoke of CC (data for comparison taken from published studies). At the higher temperature (300^{o}C) the surface area of particles in the EN(N)DS aerosol were lower those at 37^{o}C, both with and without nicotine (3.35 ± 1.5 and 2.48 ± 0.8 x 10^{10} nm^{2} cm^{-3} respectively).

22. The surface area of particles from second-hand EN(N)DS aerosol, were considerably lower than in mainstream EN(N)DS aerosol for all combinations of parameters, i.e. at 37^{o}C, with and without nicotine (5.90 ± 1.4 and 5.16 ± 0.8 x 10^{7} nm^{2} cm^{-3} respectively) and 300^{o}C with and without nicotine (5.32 ± 1.4 and 3.51 ± 0.8 x 10^{7} nm^{2} cm^{-3} respectively).

23. To summarise, the authors showed that particle number and surface area were higher in aerosols from EN(N)DS with nicotine for both mainstream and

[3] A paper characterising the EN(N)DS aerosol droplet particle fraction has been reviewed by the COT (TOX/2017/49).

second-hand scenarios. For EN(N)DS aerosol with nicotine, a higher average particle number with lower surface area was found when compared to mainstream CC smoke.

24. Received particle doses per puff were calculated from the generated and published data for both mainstream EN(N)DS aerosol and CC smoke for males and females. The surface area received was higher in males than females but remained comparable across cigarette types (for males: $5.6 \times 10^{2} – 1.1 \times 10^{3}$ and 5.42×10^{-1} mm^2 $puff^{-1}$ for CC and EN(N)DS, respectively; for females: $4.5 \times 10^{2} – 9.3 \times 10^{-2}$ and 4.93×10^{-1} for CC and EN(N)DS, respectively). The received PM_{10} content per puff was comparable in males and females and lower in EN(N)DS aerosol than in CC smoke (for males: $3.4 \times 10^{-2} – 6.3 \times 10^{-2}$ and 2.4×10^{0} mg $puff^{-1}$ for CC and EN(N)DS, respectively; for females: $3.4 \times 10^{-2} – 5.6 \times 10^{-2}$ and $2.17x\ 10^{0}$ mg $puff^{-1}$ for CC and EN(N)DS, respectively).

25. ELCR values (particle specific) were calculated for males and females on the basis of actual smoking habits, i.e. number of CC and EN(N)DS per day, puff number and duration and years of smoking.

26. The ECLR values for mainstream aerosol from EN(N)DS with and without nicotine were calculated as 7.26×10^{-6} and 7.3×10^{-6} respectively for males, and 6.28×10^{-6} and 6.11×10^{-6} for females. These values correspond to a lung cancer incidence of 0.6 new cases per 100,000 population. This compares to a particle-specific ECLR in the Italian general population of $2 – 6 \times 10^{-1}$ related to CC use.

27. For second-hand CC and EN(N)DS aerosol, ECLR values with and without nicotine were 2.7×10^{-8} and 1.29×10^{-8} in males and 2.62×10^{-8} and 1.24×10^{-8} in females respectively. These values correspond to a lung cancer incidence of between 0.001 and 0.003 new cases per 100,000 population.

28. In summary, the authors reported that the particle-specific ELCR associated with mainstream aerosol exposure from EN(N)DS is two orders of magnitude higher than that of second-hand EN(N)DS aerosol exposure. ELCR are also higher for nicotine-containing aerosols, in comparison with non-nicotine containing aerosols, and for male users when compared with females. The authors conclude that the ELCR evaluated in the study for mainstream EN(N)DS aerosol is lower than the target limit of 1×10^{-5} proposed by the WHO, and the target risk range of 10^{-6} to 10^{-4} from the US EPA, to be ‘safe and protective of public health’.

29. The contribution of each (perceived) hazardous component of EN(N)DS aerosol to the ELCR was also examined:

- Cadmium had the greatest contribution to the ELCR in EN(N)DS aerosol, with and without nicotine, and in CC smoke, contributing 42.2%, 63.9% and between 0% and 17%, respectively;

- NNK had the second largest contribution, explaining why the presence of nicotine *per se* increased the ELCR, with contributions of 27.9%, and between 69 and 88% for EN(N)DS aerosol and CC smoke, respectively.

- Arsenic, nickel and NNN were estimated to contribute 20.2%, 7.8%, and 1.7% in EN(N)DS aerosol with nicotine; 21.2%, 14.9%, and 0% for EN(N)DS aerosol without nicotine; and between 2 and 4%, 0%, and between 8 and 9% for CC smoke, respectively.

30. Taking the calculated ELCR into consideration, the authors conclude that the use of EN(N)DS as an alternative to CC significantly reduces the risk of developing lung cancer (for the Italian population) from 4×10^{-1} to around 7×10^{-6}. In addition, exposure to second-hand aerosol from EN(N)DS is associated with a negligible increment in lung cancer cases. Higher risks are associated with nicotine containing aerosols due to the presence of NNK and NNN.

31. In recognising current issues with the assessment of the relative harm of aerosols from different vaporised nicotine products (VNPs), Stephen (2018) aimed to derive a procedure that assigns a single latent variable (potency) that reflects carcinogenic risk, to an emission data set. In the first step of their methodology, cancer potencies of various nicotine-delivering aerosols were modelled using published chemical analyses of emissions and their associated inhalation unit risks. Secondly, the calculated potencies were compared using a conversion procedure for expressing smoke and EN(N)DS vapours in common units. In the third step, lifetime cancer risks were calculated from the derived potencies using daily consumption estimates.

32. To enable the modelling, concentrations of several major carcinogens present in CC smoke and in VNP 'vapour' (from a prototype heat-not-burn device, and EN(N)DS devices including early-generation disposables, second-generation clearomisers and cartomisers and third-generation modules and tanks) were obtained from various published literature. Where available, data on EN(N)DS coil resistance and battery voltage were also collated. The resulting data set contained 93 analyses divided into three subsets, namely: the 'Goniewicz subset' used as a benchmark containing 12 EC samples, with analysis for 7 carcinogens (carbonyls, VOCs, nitrosamines and metals); the 'organics subset' was divided into two with the 'variable power (organic) subset' providing concentrations of some organic compounds (formaldehyde, acetaldehyde and, in some studies, VOCs) in conjunction with data on coil heating effects and constituted 32 analyses; the remaining 'organics only' subset provided data for the above organics only and comprised 48 analyses. Carcinogen emissions from an unheated medical nicotine inhaler device were considered to represent an 'accepted' level of exposure and uncontaminated air a reference baseline.

33. The compounds that were assessed comprised: acetaldehyde; formaldehyde; acrylonitrile; benzene; 1-3-butadiene; 2-amino-naphthalene; 4-amino biphenyl, benzo(a)pyrene; NNN; NNK; cadmium; lead; chromium; nickel and arsenic. These

are classified by IARC as either *human carcinogens* (Group I) or *possible human carcinogens* (Group 2B). The mean potency ratio of EN(N)DS relative to CC smoke was reported as 1.8×10^{-3}. The aerosols from all sources tested formed a spectrum of relative cancer potencies that spanned five orders of magnitude (around $10^{0} – 10^{-5}$); lowest relative potencies were assessed as ambient air and highest potencies as CC smoke. There was a large variation in potency calculated for EN(N)DS emissions which spanned most of this range. Although the majority of potencies for EN(N)DS were <1% of that for tobacco smoke (around 10^{-3} of the potency of tobacco smoke), these were two orders of magnitude higher than that of the medicinal nicotine inhaler (around 10^{-4} that of CC smoke).

34. A small number of the sub-sets assessed (organics-only and variable power subset) had noticeably higher potencies. These tended to be associated with high levels of carbonyls generated when excessive power is delivered to the atomiser coil.

35. The predominant carcinogens within the potency estimates were found to differ for the different devices. For CC, the authors state that 1,3-butadiene and acrylonitrile accounted for 75% of the cancer potency, whereas for EN(N)DS, formaldehyde and acetaldehyde accounted for >95% of organic compound contribution to cancer potency; cadmium was also found to influence potency but was not present in all devices tested.

36. The potential for cancer potencies to be positively influenced by the applied voltage to VNP devices was also highlighted by the authors. It was considered that carbonyl potency may be enhanced by an increased rate of heat energy transfer at the coil, although no consistent relationship was seen in the studies assessed.

37. Calculated mean lifetime cancer risks (for 15 cigarette equivalents per day for a lifetime[4]) were found to decline in the following sequence: CCs >> heat-not-burn >> e-cigarettes (normal power) ≥ nicotine inhaler; 2.4×10^{-2}, 5.7×10^{-4}, 9.5×10^{-5} and 8.9×10^{-6} respectively.

38. When compared with CC smoking, the authors state that the relative risks are lower for the other devices (0.024, 0.004 and 0.0004 for heat-not-burn, EN(N)DS and nicotine inhaler respectively). However, in comparison with the medical use device, the authors report a higher relative risk (11, 64 and 2700 for EN(N)DS, heat-not-burn CC respectively).

39. The authors concluded that optimal combinations of device settings, liquid formulation and vaping behaviour normally result in EN(N)DS emissions with much less carcinogenic potency than CC smoke. Nevertheless, they highlight the potential for increased risks when EN(N)Ds products are not used according to manufacturer's guidance.

[4] 15 traditional cigarettes per day or 15 heat-not-burn sticks or 30L e-cigarette liquid (normal power) or 30L nicotine liquid from a nicotine inhaler.

40. The authors note that the carcinogenic risks calculated in the study refer to chemical risk only and not to other factors such as small particle size. In addition, aggregate and/or synergistic risks were not taken into account using their methodology. A major limitation with the data used was the absence of measurements for metals[5] which were shown to have a large influence on the unit risk value, and this may have resulted in an underestimate of cancer potency values.

41. In conclusion, the study showed, using their methodology, that a considerable range of cancer risks can be derived from currently available emissions data for VNPs. Of particular note is the requirement for a better understanding of the influence of carbonyls and metals on cancer risk for these devices. This may subsequently lead to better control of exposure to these substances in aerosols through device and e-liquid formulation design and vaping behaviour.

42. As part of the recent NAS report, a systematic review of currently available evidence relating to a potential association between EN(N)DS use and carcinogenesis was carried out. The authors comment that due to the relatively recent introduction of these products and poor design of many of the studies currently available, there is a paucity of evidence on the long-term effects on cancer outcomes. As such, much of that reviewed is based on existing evidence regarding the carcinogenic potential of the major components of EN(N)DS products, for example, nicotine (NAS, 2018).

43. The authors considered that there are many biologically plausible pathways by which components of EN(N)Ds products could, theoretically, influence the development of cancer. It was considered that evidence showing the ability of EN(N)DS aerosols to form ROS and/or be converted to DNA binding reactive intermediates was of particular relevance to the outcome of chemical carcinogenesis. In addition, evidence showing the cytotoxic potential of EN(N)DS aerosols that may contribute to tissue repair and mitogenic response was also highlighted as an important pathway for chemically induced cancers.

44. The major findings of the review can be summarised as being:

- There are few epidemiology studies that allow meaningful interpretation about cancer or intermediate cancer endpoints and those that have been carried out are of poor quality. They do not provide an evidence base to allow even preliminary associations between the use of EN(N)DS products and the risk of cancer in humans to be interpreted.

- *In vivo* animal studies provide *limited evidence* of an increased risk of cancer following long-term use of EN(N)DS products, based on the intermediate cancer biomarker, 8-OHdG. This statement is cautioned by the authors as the utility of 8-OHdG as a predictive biomarker for carcinogenesis is limited.

[5] A paper concerning metal exposure from EN(N)DS aerosol has been reviewed by the COT (TOX/2018/15).

- No adequate long-term (2-year) animal studies of exposure to EN(N)DS aerosol were identified during the systematic review.

- There is *limited evidence* that the aerosol from EN(N)DS products is mutagenic or can cause DNA damage in humans, animal models and human cells *in vitro*.

- Substantial evidence is available that a number of chemicals present in the aerosols from EN(N)DS products cause DNA damage and are mutagenic (for example, formaldehyde and acrolein), supporting the biological plausibility of an increased risk of cancer through their use. However, the levels of exposure to these through EN(N)DS product use remains to be determined.

Questions for the Committee

45. Members are asked to consider this paper and in particular:

    i. Is the Committee able to comment on the absolute and relative risks of carcinogenicity of E(N)NDS compared to conventional cigarettes?

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

Abbreviations/Glossary

Cartomiser:	Combination of cartridge and atomiser within e-cigarette device.
CC:	Conventional cigarettes
Clearomiser:	Transparent version of cartomiser e-cigarette device
CYP:	Cytochrome P450
ELCR:	Excess lifetime cancer risk
EN(N)DS, 'e-cigarettes':	Electronic nicotine (and non-nicotine) delivery systems
EPR:	Electron paramagnetic resonance
FRAP:	Ferric reducing antioxidant power
NNK:	Nicotine-derived nitrosamine ketone
NNN:	*N*-nitrosonornicotine
PAH:	Polycyclic aromatic hydrocarbon
PG:	Propylene glycol
ROS:	Reactive oxygen species
VG:	Vegetable glycerine
VOC:	Volatile organic compound
VNP:	Vapourised nicotine product

References

Canistro, D., Vivarelli, F., Cirillo, S. et al (2017) E-cigarettes induce toxicological effects that can raise the cancer risk. Scientific Reports, 7(1): 2028.

Fuller, T.W., Acharya, A.P., Meyyappan, T. et al (2018) Comparison of Bladder Carcinogens in the Urine of E-cigarette Users Versus Non E-cigarette Using Controls. Scientific Reports, 8(1): 507.

National Academies of Sciences, Engineering, and Medicine (2018) Public health consequences of e-cigarettes. Washington, DC: The National Academies Press. Available at: http://www.nas.edu/hmd/Reports/2018/public-health-consequences-of-e-cigarettes.aspx [accessed June 2018].

Scungio, M., Stabile L., Buonanno, G. (2018) Measurements of electronic cigarette-generated particles for the evaluation of lung cancer risk of active and passive users. Journal of Aerosol Science, 115: 1-11.

Stephens, W.E. (2018) Comparing the cancer potencies of emissions from vapourised nicotine products including e-cigarettes with those of tobacco smoke. Tobacco control, 27, 10-17.

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (EN(N)DS – e-cigarettes) – overview of available data on carcinogenicity.

Search strategy

Two searches were carried out in both SCOPUS and PubMed. Search terms in each database are as follows:

- Genotoxicity

  Scopus
  (TITLE-ABS-KEY ("e-cig*" OR "electronic cigarette*" OR "electronic nicotine delivery system*") AND TITLE-ABS-KEY (genotox* OR mutagen* OR "genetic tox")): 30 refs.

  PubMed
  ((("e-cig*" [Title/Abstract] OR "electronic cigarette*" [Title/Abstract] OR "electronic nicotine delivery system*"[Title/Abstract])) AND (genotox* [Title/Abstract] OR mutagen* [Title/Abstract] OR "genetic tox*"[Title/Abstract])) AND english[Language]: 12 refs.

- Carcinogenicity

  Scopus
  (TITLE-ABS-KEY ("e-cig*" OR "electronic cigarette*" OR "electronic nicotine delivery system*") AND TITLE-ABS-KEY (carcin*)): 145 refs.

  PubMed
  ((((("e-cig*" [Title/Abstract] OR "electronic cigarette*" [Title/Abstract] OR "electronic nicotine delivery system*"[Title/Abstract])) AND (carcin* [Title/Abstract]))) AND english[Language]: 38 refs.

All papers were screened for relevance by assessing the title, keywords and abstract. Papers that reported data of interest regarding the genotoxicity or carcinogenicity of E(N)NDS were selected. Papers were then separated into those relevant for COM (presented here) and for COC (to be presented at the July COC meeting).

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

CC/2018/01 Annex 2

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (EN(N)DS – e-cigarettes) – overview of available data on carcinogenicity.

Chapter 10 National Academies of Sciences, Engineering, and Medicine (2018) Public health consequences of e-cigarettes. Washington, DC: The National Academies Press. Available at: http://www.nas.edu/hmd/Reports/2018/public-health-consequences-of-e-cigarettes.aspx [accessed June 2018].

This document is attached. It is not being made publicly available for copyright reasons.

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

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes) – update of available data on carcinogenicity.

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

Search strategy

Two searches were carried out in both SCOPUS and PubMed. Search terms in each database are as follows:

Scopus

(TITLE-ABS-KEY (“e-cig*” OR "electronic cigarette*" OR "electronic nicotine delivery system*") AND TITLE-ABS-KEY (carcin*)) AND (EXCLUDE (LANGUAGE , "German") OR EXCLUDE (LANGUAGE , "French") OR EXCLUDE (LANGUAGE , "Italian") OR EXCLUDE (LANGUAGE , "Chinese") OR EXCLUDE (LANGUAGE , "Danish") OR EXCLUDE (LANGUAGE , "Polish")): 66 refs

PubMed

(((e-cig* [Title/Abstract] OR "electronic cigarette*" [Title/Abstract] OR "electronic nicotine delivery system*"[Title/Abstract])) AND (carcin* [Title/Abstract])) AND english[Language]: 6 refs

Papers that were identified from the search in 2018 were excluded. All remaining papers were screened for relevance by assessing the title, keywords and abstract. Reviews papers were excluded. Papers that reported data of interest were selected.

NCET at WRc/IEH-C under contract supporting the PHE COC Secretariat
October 2019

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Minutes of the meeting held at 10.30am on Thursday 12th July 2018 at Public Health England, CRCE, Chilton, Didcot, Oxon, OX11 0RQ.

ITEM 4: Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes) – overview of available data on carcinogenicity (CC/2018/01)

19. No interests were declared for this item.

20. The COT was considering the potential toxicological risks of electronic nicotine (or non-nicotine) delivery systems (E(N)NDS). A paper (TOX/2018/16) had been presented at the COT, in which a literature search for evidence on genotoxicity and carcinogenicity had been undertaken and full lists of publication titles retrieved were presented. After follow-up analysis of the abstracts, it was agreed that the COM and the COC should consider the available papers on genotoxicity and carcinogenicity, respectively. The aim was for the COC (and COM) to assess absolute and relative risks from E(N)NDS compared to conventional cigarettes, and if feasible, to heated tobacco products.

21. Members raised concern around the use of flavourings in E(N)NDS products and queried whether there was an 'approved' list for use in such products, as there was for addition to conventional cigarettes and food flavourings. The extent of carcinogenicity testing of the flavourings via the inhalation route was considered to be a potential issue, with most testing presumed to be by the oral route. Diacetyl butter flavour was highlighted as an example that should be flagged up to COT as of concern for potential carcinogenicity.

22. Thermal decomposition of flavourings and other materials within E(N)NDS products was considered to be of potential concern. Members commented that where thermal decomposition within E(N)NDS products had been compared to conventional cigarettes, it was unclear how the values had been derived. It was difficult to reach a conclusion on the relative risks from thermal decomposition in E(N)NDS compared to conventional cigarettes.

23. The Committee was informed that there was guidance available from WHO regarding use parameters for E(N)NDS to minimise the risks to the user. Although it was acknowledged that this was aimed at regulators and industry, Members suggested consideration be made of whether this could be modified for dissemination for customers and users of the devices.

24. It was noted that the risk to new users taking up the use of E(N)NDS products had not been considered in the papers. One of the papers had carried out a comparison of the risk associated with using conventional cigarettes, heat-not-burn products and E(N)NDS products. The members considered that the risk for tobacco-containing products was implicit to the user as tobacco doesn't need to be heated to

be carcinogenic. For E(N)NDS products, the available evidence suggested that nicotine itself was not a carcinogen.

25. There was some discussion on the potential risks to bystanders from exhaled aerosols and whether there was a difference between second hand smoke from conventional cigarettes when compared to E(N)NDS products. It was noted that only limited data were available on this topic.

26. One member noted that the COM had also reviewed mutagenicity studies as part of the COT review. They considered that although there was a breadth of evidence reported, those studies conducted to OECD Test Guidelines showed negative results and these had been sponsored by industry. The non-test guideline studies generally reported positive results, but they did not show consistency and had not been repeated by other investigators. COM members had also expressed concern that some studies reported genotoxicity only when wider toxic effects were observed. The COM concluded that the limited evidence base did not indicate any specific mutagenic risks from E(N)NDS that were not observed with conventional cigarette products. However, COM members considered that greater consistency and demonstrable reproducibility in both product, exposure and methodologies were needed before any view could be taken on absolute risks of E(N)NDS products.

27. The COC concluded that relative risk of E(N)NDS compared to conventional cigarettes appeared to be lower, but there was still some risk associated with the chemicals and particles in the emissions from E(N)NDS. This risk should be emphasised to new users. In addition. Members concluded that the possibility of bystander effects should also be considered.

28. A brief discussion on the possible value of co-ordinating animal studies on E(N)NDS products in the UK in the future led to the conclusion that these would not be very useful for carcinogenicity assessment, as animal models had not been good proxies for the human health effects of cigarettes.

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes) – update of available data on carcinogenicity.

Paragraphs describing the report of Lee et al. (2018), as taken from previous COT and COM discussion papers, are reproduced below.

COT (TOX-2018-24)

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 FVBN 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. O6-methyl-deoxyguanosine (O6-medG) adduct and the 1,N2-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 O6-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.

COM (MUT-2018-08)

In a complex in vivo/in vitro study, Lee et al. (2018) investigated E(N)NDS aerosols in terms of their potential to affect the nitrosation of nicotine with the subsequent formation of nitrosamines. DNA damage, induced by nitrosamines, was measured in the organs of FVBN mice exposed to either filtered air (control group) or aerosols of

the nicotine-containing E(N)NDS, NJoy, generated by a smoking machine. According to the authors, exposure was equivalent to the dose and duration of light E(N)NDS use for 10 years; namely 10 mg/ml, 3 hours/day, 5 days/week for 12 weeks.

On examination of organs, significant numbers of O-methyldeoxyguanosine adducts were detected in the heart, liver, bladder and, particularly, the lung (3-8-fold higher) of the E(N)NDS aerosol-exposed mice. Further adducts were also detected based on aldehyde-derived cyclic 1,N 6-propano-dG, which were noted by the authors as the main adducts induced by exposure to traditional tobacco smoke in the mouse (not measured in this study). These adducts were also most abundant in the lungs. It was concluded that DNA damaging agents were present in the E(N)NDS aerosol. Further analysis showed that levels of XPC and OGG1/2, enzymes responsible for nucleotide and base excision repair, were reduced in the lung tissue of E(N)NDS aerosol exposed mice.

In a parallel study, Lee et al. (2018) conducted a series of assays in human bronchial epithelial (BEAS-2B) and urothelial cells (UROtsa) with nicotine and the metabolites of inhaled nitrosamines, N-nitrosonornicotine (NNN) and nicotine-derived nitrosamine ketone (NNK), to compare effects with those observed in E(N)NDS aerosol exposed mice. Nicotine, NNN and NNK induced the same adducts in vitro, as seen in vivo following E(N)NDS aerosol exposure. DNA repair was also reduced in vitro. Using a SupF mutation system, NNK and nicotine enhanced spontaneous, UV- and H2O2-induced mutation frequency and greatly induced anchorage independent growth of human lung and bladder cells. The authors concluded that exposure to E(N)NDS aerosol damaged DNA in mouse lung and bladder and that this process could involve nicotine and products of nitrosation.

NCET at WRc/IEH-C under contract supporting the PHE COC Secretariat
October 2019