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COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

Potential toxicological risks from electronic nicotine (or non-nicotine) delivery systems (e-cigarettes). Overview of available data on genotoxicity.

Background

1. The COT is currently considering the potential toxicological risks of electronic nicotine (or non-nicotine) delivery systems (E(N)NDS or 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 COM and COC should consider the available papers on genotoxicity and carcinogenicity, respectively. The aim is for COM (and COC) 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].

3. A total of 178 references were retrieved from the initial searches and screened for relevance to COC and COM. Of these, 14 papers were identified as needing consideration by COM. Details of the search string are provided in Annex 1. These papers are discussed in the following sections, categorised using the endpoints of assessment, and are available in full in Annex 2.

Regulatory genotoxicity assays

4. In a study by Misra *et al*. (2014), a range of commercial E(N)NDS liquids (commercial blu E(N)NDS containing glycerol-based e-liquids, with and without nicotine and two market leader flavours) and pad-collected particulate matter from aerosols from E(N)NDS were tested in a battery of *in vitro* assays for cytotoxicity, mutagenicity, genotoxicity and inflammation. Findings were compared with pad-collected smoke condensates from tobacco burning cigarettes (Kentucky 3R4F, 1R5F and Marlboro Gold), extracts of smokeless tobacco products (SLT; Marlboro Snus, Copenhagen Snuff) and a nicotine replacement therapy product (NRT; Nicorette lozenge) tested under the same conditions. Cytotoxicity (measured using the neutral red assay) and inflammation (interleukin (IL)-8 levels) were determined in human lung epithelial carcinoma cells (A549). Mutagenicity was assessed using *Salmonella typhimurium* strains TA98 and TA100 (Ames assay) and genotoxicity determined through the frequency of micronuclei (MN) in CHO-K1 cells.

5. The authors reported that no cytotoxicity or induction of IL-8 release was observed in A549 cells following exposure to any of the E(N)NDS liquids or aerosols, SLT or NRT products. In addition, negative results were observed in both strains in the Ames assay and there was no increase in the frequency of MN due to any of the test compounds (liquids or aerosols). In contrast, the pad-collected particulate matter samples from all tobacco cigarettes showed a dose-dependent induced IL-8 release in A549 cells, indicating an inflammatory response. This induction was seen at doses of particulates 20 times lower than the maximum E(N)NDS aerosol concentration at which no induction was observed.

6. The mutagenic potential of the aerosol from an E(N)NDS device containing tobacco-flavoured e-liquid was evaluated using the Ames test with strains TA98 and TA100 (Thorne et al. 2016) and TA98, TA100, TA104 and *E. coli* WP2 *uvr*A with and without metabolic activation (Thorne et al. 2018) carried out according to OECD Guideline 471. In the first study, aerosol from the E(N)NDS was either collected on a filter pad as particulate matter (aerosol collected matter (ACM)) which was dissolved in a solvent or as freshly generated E(N)NDS aerosol assayed as an air-agar interface. Comparisons with mainstream smoke from a Kentucky reference 3R4F

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

cigarette prepared according to Health Canada standard protocols were made (Thorne et al. 2016). This delivers a higher 'puff' volume over a shorter period of time (24 minutes).

7. Both the E(N)NDS ACM and freshly generated E(N)NDS aerosol were found to be non-mutagenic in the Ames test using strain TA98 and TA100. The reference cigarette 3R4F was positive in both strains (Thorne et al. 2016). The first study utilised an aerosol generated at the agar interface and diluted to give a range of concentrations corresponding to numbers of 'puffs' which was validated by analysis of nicotine concentration. In the second study, the undiluted E(N)NDS aerosol was assayed as the air-agar interface. No mutagenic activity was observed in any of the strains used, both with or without metabolic activation. The authors noted that although not tested in this experiment, the 3R4F cigarette had previously been shown to be positive in these strains under the same test conditions (Thorne et al. 2018).

Oxidative stress and oxidative DNA damage

8. In an *in vivo* study, groups of Sprague-Dawley rats (10 animals per exposed and non-exposed control group) were exposed to vapour from a commercial E(N)NDS product described as "Essential cloud, red fruit flavour" by inhalation (Canistro et al. 2017). Authors described a number of volatile compounds (mainly nicotine, propropylene glycol and vegetable glycerine as well as minor compounds and flavours; 1,2-propanediamine, acrolein, indole, acetol, 3-hexene-1-ol, diacetyl, propylene glycol, 1-methoxy-2-propyl acetate, methyl propionate, propanoic acid, 1-methylpropyl ester) that were detected in the chambers during exposure to the E(N)NDS aerosol. Animals were exposed, in a chamber, to a total of 1 ml/day containing 18 mg/ml of nicotine and consisting of 11 cycles/day for 5 consecutive days/week for 4 weeks. One cycle was a 17 second 'puff'. The rats were euthanised and the lungs, whole blood, urine and plasma collected for a range of metabolic and genotoxic assays and the results are outlined below.

9. When compared to unexposed controls, an increase was also observed in levels of the oxidative DNA lesion, 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the lungs. Further analysis also showed DNA damage in leucocytes (as measured by the Comet assay) and an increase in immature micronucleated reticulocytes. Urine collected from the E(N)NDS aerosol-exposed rats was shown to induce an increased incidence of revertants in strains TA100 (base substitutions) and YG1024 (frameshift mutations) of *Salmonella typhimurium*.

10. The authors reported an increase in cytochrome P450 (CYP) 1A1/2, CYP2B1/2 and CYP3A and a significant increase in free radical levels (observed using an electron paramagnetic resonance technique) in the lungs. The authors suggested that such increases in CYP enzymes might alter the metabolism of

procarcinogens present in E(N)NDS vapours and potentially predispose individuals to enhanced cancer risk. This was accompanied by a significant decrease in levels of the antioxidant enzymes, catalase, diaphorase and superoxide dismutase, and glutathione-S-transferases. Systemic antioxidant capacity was significantly reduced in the lungs with a similar, but not significant reduction, observed in plasma. This decrease appeared to be inversely correlated to levels of carbonyl residues in the E(N)NDS aerosol exposed rats.

11. A further study into the potential oxidative effects of E(N)NDS product exposures was conducted by Ganapathy *et al*. (2017). In this investigation, five distinct extracts were prepared from two devices: Njoy traditional flavor (12 and 18 mg/ml nicotine) and eGo-T Desert Sands Flavor (12 and 18 mg/ml nicotine plus a nicotine-free liquid). Traditional tobacco smoke extracts were prepared from Marlboro 100 using methods based on Health Canada Intensive (HCI) smoking standard conditions. *In vitro* assays were conducted on human epithelial normal bronchial cells (Nuli1) and human oral squamous cell carcinoma (UM-SCC-1). For short-term exposures cells were treated for one hour with 1, 10 and 100 puffs/5L while for chronic exposure, cells were treated every other day for 2 weeks with 10 puffs/5L (this dose was also used for traditional smoke extract and had previously been shown to cause significant DNA damage under the conditions used).

12. DNA damage was quantified using a primer-anchored DNA damage detection assay (q-PADDA) within the transcribed and non-transcribed strands of *p53* (used as this is the most frequently mutated gene in human cancer) and through measurement of the levels of 8-OHdG. Cellular oxidative stress was assessed by the detection of reactive oxygen species (ROS), total cellular antioxidant activity (TAC) and cell viability (using the tetrazolium, MTT assay) and protein and RNA expression was measured using Western blot and RT-PCR, respectively. Using q-PADDA, E(N)NDS aerosol extracts were shown to induce DNA damage in a dose-dependent manner that was independent of nicotine content. However, the DNA damage observed was significantly less than that seen with traditional cigarette smoke. DNA damage from E(N)ND aerosol extracts, as indicated by levels of 8-OHdG, was similar to that from traditional cigarette smoke and was accompanied by a significant increase in ROS and decreased TAC and expression of DNA glycosylase (OGG1), an enzyme essential for the removal of oxidative DNA damage.

13. Lerner *et al.* (2016) investigated the potential toxic effects of E(N)NDS aerosols on mitochondrial systems in human lung fibroblasts (HFL-1) *in vitro*. A liquid-air interface system was used, and the E(N)NDS studied was nicotine-containing Lorillard Blu Classic Tobacco with 4 second puffs every 30 seconds for varying lengths of time (5, 10, 15, 20 minutes). Fluorescence techniques were used to determine mitochondrial superoxide and membrane potential, immunoblotting techniques to determine electron transport complex (ETC) proteins, the Comet assay

to assess DNA fragmentation and ELISA for the measurement of cytokines, interleukin-6 (IL-6) and IL-8.

14. HFL-1 cells exposed to E(N)NDS aerosol showed increased production of mitochondrial ROS when compared to 'air control' cells. Measurement of the expression of the ETC protein, Nqo1 indicated an increase in Antioxidant Response Element (ARE) inducible protein after 10 and 20 minutes' exposure, suggesting that E(N)NDSs aerosol trigger ARE responsive genes. Copper nanoparticles incubated with NFL-1 cells also increased ROS and as copper has been detected in E(N)NDS aerosols, the authors suggested that metal particles might be a mediator of the observed mitochondrial ROS generation. The E(N)NDS aerosol also affected electron transport chain proteins in these cells as shown by a decrease in COXII levels. Longer (>5-minute) exposures resulted in a significant increase in DNA fragmentation; There seems to be a limit to the increase in DNA damage with time; 75% at 10 min and 57% at 15 min and this is accompanied by an increase in likelihood of the air controls showing DNA fragmentation. An increase in the pro-inflammatory cytokines, IL-6 and IL-8 were also apparent with longer exposure times. From these findings, the authors concluded that E(N)NDS aerosol exposure elicited biological effects associated with increased mitochondrial ROS and genotoxic stress and an inflammatory stress response.

DNA damage and cytotoxicity

15. A study by Yu *et al*. (2015), and abstracted by Holliday *et al*. (2016) investigated the cytotoxicity and genotoxicity of E(N)NDS "vapour", following short- and long-term exposure, on a panel of normal epithelial (HaCat) and head and neck squamous cell carcinoma (HNSCC) cell lines (HN30 and UMSCC10B derived from the oropharynx - primary laryngeal tumour and metastatic lymph node, respectively). Nicotine-containing and nicotine-free versions of the E(N)NDS, V2 'Classic Tobacco' and VaporFi 'Red-American Tobacco' e-liquids were used to generate aerosols and compared with smoke from a traditional tobacco-containing cigarette, Marlboro Red filter. Aerosols were pulled through media, the extract filter-sterilised and incubated with the cells for between 48 hours and 8 weeks, with media being replaced every 72 hours. Owing to the high toxicity of the cigarette smoke extract, the cells were only treated for 24 hours.

16. Exposed cells were analysed for cytotoxicity using flow cytometry, trypan blue exclusion and clonogenic assays, and for genotoxicity through DNA strand breaks using a neutral Comet assay and ϒH2AX[2] immunostaining. E(N)NDS aerosols caused significantly reduced cell viability and clonogenic survival along with increased rates of apoptosis (measured by Annexin V binding) and necrosis both with and without nicotine. Increased Comet tail length and accumulation of ϒH2AX foci indicated an increase in DNA double strand breaks. Exposure to traditional

[2] phosphorylation of a nuclear protein representing a response to DNA double strand breaks

cigarette smoke was associated with a higher number of double-strand breaks than any of the E(N)NDS aerosols.

17. Thorne *et al.* (2017) also investigated the potential effects of exposure to E(N)NDS aerosols on double-strand DNA damage in human lung epithelial cells (BEAS-2B) using the ϒH2AX assay; traditional cigarette smoke from Kentucky 3R4F was used as a comparison. Aerosols were generated at the agar interface and diluted to give a range of concentrations corresponding to numbers of 'puffs' which was validated by analysis of deposited particulate mass and nicotine concentration. Cell viability was measured using nuclear DNA staining (Hoechst dye). Aerosol exposures were chosen to be below cytotoxic levels except for the highest dose of traditional cigarette smoke. Clear dose-response DNA damage was observed with increasing concentrations of traditional cigarette smoke, up to cytotoxic levels. However, in contrast to the study of Yu *et al*. (2015) outlined above, the E(N)NDS aerosols did not induce double-stranded DNA damage at exposure doses 12-28 times the concentrations of cigarette smoke.

18. Welz *et al*. (2016) studied the effect of E(N)NDS aerosol on mucosal tissue cultures ("a spheroidal *in vitro* model with biotransformative activity") assembled from fresh healthy oropharyngeal mucosa. The three E(N)NDS liquids used in the study were apple, cherry and tobacco flavours (Happy Liquid GmbH) and all contained nicotine (12 mg/ml). Aerosols were incubated at three different concentrations with the tissue cultures for 24 hours or for 2.5 hours on 5 sequential days. Cytotoxicity was measured using a MTT assay and DNA damage assessed using the Comet assay. The authors reported that aerosols from E(N)NDS liquids were cytotoxic. Whilst the fruit liquids showed significantly increased DNA fragmentation indicative of damage, for the tobacco-flavoured liquid the DNA damage was only moderate, but still significant.

19. 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.

20. On examination of organs, significant numbers of O^6-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^2-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.

21. 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 H_2O_2-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.

22. Tommasi *et al*. (2017) used two validated *in vitro* model systems to investigate whether E(N)NDS aerosol induces mutations in mouse and human cells. Three E(N)NDS products were studied: blue cigs, NJoy and V2 Cigs, all containing nicotine. A smoking machine was used to produce an aerosol which was evaporated and dissolved in a solvent and extract concentrations expressed as total puff equivalents (number of puffs of aerosol dissolved per ml of solvent). Transgenic mouse fibroblasts were utilised to determine whether exposure to E(N)NDS aerosol was associated with the induction of mutagenesis in the reporter gene, *cII*. In addition, the authors treated the pSP189 plasmid with E(N)NDS aerosol extract and transfected the plasmid into human fibroblast cells. Cells were screened for the induced mutations in the supF gene. Two tobacco carcinogens, benzo(a)pyrene (B[a]P) and 4-aminobiphenyl (4-ABP) were used as positive controls.

23. The E(N)NDS aerosol extracts did not induce mutagenicity in *cII*. Conversely, treatment of the same cells with B[a]P and 4-ABP resulted in statistically significant increases in the *cII* mutant frequency relative to background ($P < 0.05$). The mutation frequency in the supF gene following exposure to E(N)NDS aerosol extract was marginally, but not significantly, increased compared to the control (cells transfected with solvent-treated plasmid). In contrast, cells transfected with ultraviolet (UV)-irradiated plasmid (serving as positive control) showed a statistically significant increase in relative supF mutant frequency, which was 10-fold over the background ($P < 0.05$).

24. Behar *et al*. (2016) investigated the toxicity of a specific common constituent of E(N)NDSs, cinnamaldehyde (CAD). The authors tested 39 E(N)NDS refill liquids falling within five categories: tobacco, fruit, sweet, cinnamon and flavoured tobacco, and of these 20 contained CAD at varying concentrations. One of the E(N)NDS liquids containing a higher level of CAD, Cinnamon Ceylon, was chosen for further investigation and aerosol extracts prepared using a smoking machine (operated at 3

or 5V) to 6 total puff equivalents. The cell lines, hPF (differentiated human adult lung cell), A549 (human lung epithelial cells) and hESC (a model for early post-implantation human embryos) were exposed to 0.06, 0.2, 0.6, 2 and 6 total puff equivalents for 48 hrs. Cytotoxicity was measured by the MTT assay, effects on cytoskeleton by fluorescence imaging of DAPI staining, live cell imaging by time lapse video, and DNA damage by the Comet assay.

25. Cinnamon Ceylon aerosol extract was shown to be cytotoxic in all three cell lines, with greater cytotoxicity apparent at 5V operation when compared to 3V. Chemical analysis showed 10 chemicals detected at 5V operation which were not present in the aerosol prepared at 3V; benzyl methyl ketone, phenol, 2-acetate-1,2-propanediol, 1-phenyl-1,2-propanedione, 2,3-butanedione, α-ethyl-benzenemethanol, 4-methyl-2(5H)-furanone, 2,methyl-1,3-dioxolane, cinnamyl alcohol, 2,4-dimethyl-1,3-dioxolane-2-methanol. The remainder of the analyses were carried out with CAD rather than E(N)NDS aerosols. hPF cells showed greater sensitivity to short-term (2 hr) CAD exposure than hESC cells and were less able to recover (as measured by live cell imaging). Treatment of hPF and hESC with CAD at non-cytotoxic and 50% toxicity concentrations led to depolymerisation of microtubules and microfilaments. hESC cells exposed to non-toxic CAD concentrations showed inhibited growth but increased motility and cell death. Comet assays performed on hPF and hESC cells at non-toxic CAD concentrations showed increased DNA damage, although hESC cells recovered after 24 hours.

Preliminary models for assessing cancer risk from E(N)NDS

26. An assessment of the relative ability of E(N)NDS and traditional cigarettes to induce tumour promotion was carried out by Breheny *et al*. (2017) using the *in vitro* Bhas cell transformation assay, recently the subject of an OECD guidance document following international validation exercises. The tested products were the E(N)NDS, Vype ePen and the Kentucky reference cigarette, 3R4F which were used to generate total particulate matter/aerosol using the methods described by Thorne *et al*. in 2016 and 2018. The cytotoxicity of the E(N)NDS aerosol extracts on Bhas 42 mouse fibroblast cells was assessed in a cell growth assay using crystal violet staining and a concentration eliciting 50% relative toxicity chosen for the tumour promotion assay. The cells were treated with extract for 10 days, the media changed and left for a further 7 days after which the cells were fixed with methanol and transformed foci counted. The tumour promoter TPA was used as a positive control. The 3R4F aqueous smoke extract was shown to be highly cytotoxic and was not scored for cell transformation while the non-toxic concentration was negative in the tumour promotion assay. The aqueous extracts from the E(N)NDS aerosols was not cytotoxic even at the highest concentration and was negative in the cell transformation assay at the highest concentrations.

Summary and discussion

27. The papers outlined in this review represent studies to assess the genotoxicity of E(N)NDS liquids and aerosols undertaken in the last few years and, as such, represent early evaluations of these products. Testing has been mainly carried out using relevant *in vitro* systems such as human lung or oral cell models. These studies have often compared the toxicity of E(N)NDS liquids and aerosols with that of tobacco, rather than being an assessment of E(N)NDS products *per se.* Presently, there has been only limited *in vitro* testing using standard Ames and micronucleus regulatory tests.

28. The findings to date have been generated using a number of different products and systems and in only a limited number of studies has the constituent(s) of the product being tested been analysed in any detail. Due to the variable nature of the E(N)NDS products, there are many variable factors to consider when assessing the general potential toxicity of these as a whole, in contrast to each individual product. However, consistent findings observed with many different products and systems could yield a weight-of-evidence conclusion for E(N)NDS in general.

29. A further variable has been the physical state of the product tested which has included the original liquid (E(N)NDS liquid or 'e-liquid'), a condensate of the aerosol produced by a standard smoking machine or an air-liquid interface system where a controlled amount of the aerosol passes over the *in vitro* media (such as agar). A number of different testing systems have also been utilised to define a standard concentration, including 'puffs per hour', nicotine concentration and particulate number after collection of the particulates on a filter. In most of the studies described, cytotoxicity of the system has been investigated and non-cytotoxic (or a known toxicity e.g. 50% cytotoxicity) concentrations used to ascertain genotoxicity as a means to standardise findings.

30. There has been only limited testing on E(N)NDS completed to OECD regulatory guidelines. A number of *Salmonella typhimurium* strains (TA98, TA100, TA100, TA104) and E. coli WP *uvr*A were negative in the Ames tests reported by Thorne et al. in 2016 and 2018. There have also been a negative *in vitro* micronucleus tests in CHO cells (Thorne et al. 2018) with E(N)NDS. Tommasi *et al*. (2017), using *in vitro* assays based on the Big Blue mouse, observed no increased mutant frequency with E(N)NDS aerosol extracts.

31. In contrast, there has been a number of experimental studies, mainly *in vitro*, on E(N)NDS using a variety of relevant cell lines including lung, oral and bronchial cells. Although the results are inconsistent, double-strand DNA damage, usually assessed by the Comet assay, has been shown (Lerner et al. 2016) and *in vivo* in treated rats (Canistro et al. 2017). In a further assay measuring ϒH2AX, which is phosphorylated in response to double-strand DNA damage, negative and positive results have been reported (Yu et al. 2015, Thorne et al. 2017).

32. A number of studies have reported oxidative effects *in vitro* and *in vivo* in rats (Ganapathy *et al*., 2016; Yu *et al*., 2016). 8-OHdG adducts have been observed in treated rat lungs and in p53 DNA in cells. Oxidative stress has been observed as measured by increased reactive oxygen species and decreased antioxidant systems, including oxidative effects on ETC in mitochondria (Lerner et al. 2016). However, E(N)NDS was negative in Ames strains, TA102 and TA104 which are considered sensitive to oxidative damage (Thorne et al. 2016).

33. In conclusion, research on the potential genotoxicity of E(N)NDS is at an early stage and few robust studies have been conducted and published. The variations in product preparation, exposure systems and concentrations used mean that only hazard can begin to be assessed. While the regulatory tests on mutagenicity and genotoxicity have so far been negative, a number of studies have indicated that exposure to E(N)NDS may possibly affect DNA by oxidative effects.

Questions for the Committee

34. Members are asked to provide general comments on the paper and in particular:

i. Can the Committee comment on the methods used in the papers presented?

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

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Abbreviations

A549	Human lung epithelial cells
4ABP	4-aminobiphenyl
ACM	Aerosol Collected Matter
ARE	Antioxidant Response Element
B(a)P	Benzo(a)pyrene
BEAS-2B	Human lung epithelial cells
COC	The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment
COM	The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment
COT	The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CYP	Cytochrome P450
E(N)NDS	Electronic Nicotine (or Non-Nicotine) Delivery System
ETC	Mitochondrial Electron Transport Complex
HaCat	Human normal epithelial cell line
HCI	Health Canada Intensive standard smoking conditions
HNSCC	Human head and neck squamous cell carcinoma
HN30	HNSCC from oropharynx primary laryngeal tumour
hPF	Differentiated human adult lung cells
hESC	Model for early post-implantation human embryos
H_2O_2	Hydrogen peroxide
HFL-1	Human lung fibroblasts
IL-8	Interleukin-8
MN	Micronuclei
MTT	Tetrazolium dye exclusion assay for cytotoxicity

NRT	Nicotine Replacement Therapy
8-OHdG	Oxidative DNA lesion, 8-hydroxy-2'-deoxyguanosine
OGG1	DNA glycosylase enzyme essential for removal of oxidative damage
q-PADDA	Primer-anchored DNA damage detection assay
ROS	Reactive Oxygen Species
SLT	Smokeless Tobacco Product
TAC	Total cellular antioxidant activity
UMSCC10B	HNSCC from oropharynx metastatic lymph node.
UROtsa	Human urothelial cells
UV	Ultraviolet

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Yu, V., M. Rahimy, A. Korrapati, Y. Xuan, A. E. Zou, A. R. Krishnan, T. Tsui, J. A. Aguilera, S. Advani, L. E. Crotty Alexander, K. T. Brumund, J. Wang-Rodriguez & W. M. Ongkeko (2016) Electronic cigarettes induce DNA strand breaks and cell death independently of nicotine in cell lines. Oral Oncology, 52, 58-65.

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Potential toxicological risks from electronic nicotine (or non-nicotine) delivery systems (e-cigarettes). Overview of available data on genotoxicity.

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 COT Secretariat
March 2018

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Full literature papers

Behar, R. Z., W. Luo, S. C. Lin, Y. Wang, J. Valle, J. F. Pankow & P. Talbot (2016) Distribution, quantification and toxicity of cinnamaldehyde in electronic cigarette refill fluids and aerosols. Tobacco Control, 25, ii94-ii102.

Breheny, D., O. Oke, K. Pant & M. Gaça (2017) Comparative tumor promotion assessment of e-cigarette and cigarettes using the in vitro Bhas 42 cell transformation assay. Environmental and Molecular Mutagenesis, 58, 190-198.

Canistro, D., F. Vivarelli, S. Cirillo, C.B. Marquillas, A. Buschini, M. Lazzaretti and 17 others, (2017) *E-cigarettes induce toxicological effects that can raise the cancer risk*. Scientific Reports, 7:2028, DOI: 10.1038/s41598-017-02317-8.

Ganapathy, V., J. Manyanga, L. Brame, D. McGuire, B. Sadhasivam, E. Floyd, D. A. Rubenstein, I. Ramachandran, T. Wagener & L. Queimado (2017) Electronic cigarette aerosols suppress cellular antioxidant defenses and induce significant oxidative DNA damage. PLoS ONE, 12(5): e0177780.

Holliday, R., R. Kist & L. Bauld (2016) E-cigarette vapour is not inert and exposure can lead to cell damage. Evidence-based Dentistry, 17, 2-3. [this publication is an abstracted summary of Yu *et al*. (2015].

Lee, H. W., S. H. Park, M. W. Weng, H. T. Wang, W. C. Huang, H. Lepor, X. R. Wu, L. C. Chen & M. S. Tang (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. Proceedings of the National Academy of Sciences of the United States of America, 115, E1560-E1569.

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Thorne, D., M. Hollings, A. Seymour, J. Adamson, A. Dalrymple, M. Ballantyne & M. Gaca (2018) Extreme testing of undiluted e-cigarette aerosol in vitro using an Ames air-agar-interface technique. Mutation Research - Genetic Toxicology and Environmental Mutagenesis. 828, 46-54.

Tommasi, S., S. E. Bates, R. Z. Behar, P. Talbot & A. Besaratinia (2017) Limited mutagenicity of electronic cigarettes in mouse or human cells in vitro. Lung Cancer, 112, 41-46.

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These papers are attached. They are not being made publicly available for copyright reasons.

Secretariat
September 2017