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

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper for information on COM and COC consideration of genotoxicity and carcinogenicity risks

1. The COT are currently reviewing the possible human health effects of electronic nicotine (and non-nicotine) delivery systems (E(N)NDS, ‘e-cigarettes’). In spring 2018, it was agreed that advice should be sought from COM and COC on the absolute and relative genotoxicity and carcinogenicity risks of E(N)NDS compared to conventional cigarettes, and if possible heated tobacco products.

2. The COM and COC discussed papers on these topics at their June and July 2018 meetings, respectively. The paper and minutes from the COM discussion are attached at Annex A, and the paper and minutes from the COC discussion at Annex B, for the Committees awareness.

PHE COT Secretariat
November 2018
COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper for information on COM and COC consideration of genotoxicity and carcinogenicity risks

Discussion paper MUT/2018/08 and associated minutes.

PHE COT Secretariat
November 2018
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. 

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 uvrA 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

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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, propylene 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 (HCl) 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 YH2AX 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 YH2AX foci indicated an increase in DNA double strand breaks. Exposure to traditional 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 YH2AX 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 O6-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,N2-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 \textit{in vitro}, as seen \textit{in vivo} following E(N)NDS aerosol exposure. DNA repair was also reduced \textit{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 \textit{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, \textit{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 \textit{cII}. Conversely, treatment of the same cells with B[a]P and 4-ABP resulted in statistically significant increases in the \textit{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-ethylbenzenemethanol, 4-methyl-2(5H)-furanone, 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 uvrA 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 YH2AX, 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?

NCET at WRc/IEH-C under contract supporting the PHE COM Secretariat
June 2018
### Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A549</td>
<td>Human lung epithelial cells</td>
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<td>4ABP</td>
<td>4-aminobiphenyl</td>
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<td>ACM</td>
<td>Aerosol Collected Matter</td>
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<td>ARE</td>
<td>Antioxidant Response Element</td>
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<td>B(a)P</td>
<td>Benzo(a)pyrene</td>
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<td>BEAS-2B</td>
<td>Human lung epithelial cells</td>
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<td>COC</td>
<td>The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment</td>
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<td>COM</td>
<td>The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment</td>
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<td>COT</td>
<td>The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment</td>
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<td>CYP</td>
<td>Cytochrome P450</td>
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<tr>
<td>E(N)NDS</td>
<td>Electronic Nicotine (or Non-Nicotine) Delivery System</td>
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<td>ETC</td>
<td>Mitochondrial Electron Transport Complex</td>
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<td>HaCat</td>
<td>Human normal epithelial cell line</td>
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<td>HCl</td>
<td>Health Canada Intensive standard smoking conditions</td>
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<td>HNSCC</td>
<td>Human head and neck squamous cell carcinoma</td>
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<td>HNSCC from oropharynx primary laryngeal tumour</td>
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<td>hPF</td>
<td>Differentiated human adult lung cells</td>
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<td>hESC</td>
<td>Model for early post-implantation human embryos</td>
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<td>Hydrogen peroxide</td>
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<td>Interleukin-8</td>
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<td>MN</td>
<td>Micronuclei</td>
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<td>MTT</td>
<td>Tetrazolium dye exclusion assay for cytotoxicity</td>
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<td>Abbreviation</td>
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<tr>
<td>NRT</td>
<td>Nicotine Replacement Therapy</td>
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<td>8-OHdG</td>
<td>Oxidative DNA lesion, 8-hydroxy-2'-deoxyguanosine</td>
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<td>OGG1</td>
<td>DNA glycosylase enzyme essential for removal of oxidative damage</td>
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<td>q-PADDA</td>
<td>Primer-anchored DNA damage detection assay</td>
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<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<td>SLT</td>
<td>Smokeless Tobacco Product</td>
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<td>Total cellular antioxidant activity</td>
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<td>UMSCC10B</td>
<td>HNSCC from oropharynx metastatic lymph node.</td>
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<td>Human urothelial cells</td>
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References


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).


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.

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**
  

- Carcinogenicity

  **Scopus**
  

  **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 COM Secretariat
March 2018
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.

Full literature papers


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).]


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

Secretariat
September 2017
ITEM 5: E-CIGARETTES E(N)NDS GENOTOXICITY (MUT/2018/08)

7. The Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) is currently considering the potential toxicological risks of electronic nicotine (or non-nicotine) delivery systems (E(N)NDS). A paper (TOX/2018/16) was presented at the COT, in which a literature search and full lists of publications retrieved were presented. After follow-up analysis of the abstracts obtained, it was agreed that the COM and the COC should consider the available papers on genotoxicity and carcinogenicity, respectively. The aim was for the COM (and COC) to assess absolute risks from E(N)NDS and relative risk compared to conventional cigarettes, and if available to heated tobacco products.

8. A limited number of standard tests conducted to OECD Test Guidelines had been identified. These consisted of bacterial tests and micronuclei assays in mammalian cells, which gave negative results for E(N)NDS, while positive results were observed for conventional cigarettes. Members commented that these available OECD Test Guideline studies were conducted by or for the tobacco industry.

9. Of the other available studies, two were in vivo animal studies and the remainder in vitro studies. The two in vivo studies were a 4-week study in rats investigating genotoxicity and oxidative stress in lung, blood and urine and a 12-week study in mice assessing DNA damage and oxidative stress in various organs. The in vitro studies utilised relevant target tissue cells such as lung and oral cell systems. As a group, these studies assessed a wide range of genotoxic endpoints, including oxidative DNA damage, increase in reactive oxygen species and effects on mitochondria. There was a wide use of the comet assay in these studies.
Different exposure methods were utilised in the three in vitro studies conducted to OECD guidelines. In one study, liquid products or filtered particulates and aerosols condensed from various E(N)NDS devices were added to cell cultures. In the remaining two studies, an aerosol-media interface was utilised for direct interaction with a controlled amount of the aerosol passing over the in vitro media (such as agar).

The 'non-standard' studies (i.e. not conducted to OECD Test Guidelines) described exposure to a variety of E(N)NDS products using a number of experimental methodologies, some of which were not described in sufficient detail by the authors, making comparisons across studies difficult. A number of different systems were used to define a standard concentration for exposure, including ‘puffs’ per hour, nicotine concentration and particulate number following collection on a filter. It was noted that there was an effect of the voltage used on the E(N)NDS device, which resulted in different components in the emission. The Committee considered that it would be important for test systems to reflect exposures of users or bystanders. In addition, Members considered that standardisation of a delivery protocol would be helpful to allow for comparisons to be made across studies.

Members noted that mainly high doses had been used in the studies involving the comet assay. The DNA damage seen in these studies was associated with relatively high levels of cytotoxicity and thus could have been a consequence of toxicity rather than direct interaction with DNA. Only one comet assay appeared to provide a robust positive result. The COM also questioned the suitability of the methodology used for the measurement of 8-OHdG and the extended duration of exposure in some cell culture studies, e.g. for one study an 8-week exposure was used. Although this was associated with some genotoxicity, members considered that the extended period of exposure may have contributed to this and was not representative of human exposure to E(N)NDS which would not be continuous.

It was noted that one of the studies indicated that the carrier substance, propylene glycol, may have influenced overall toxicity. It was also noted that flavouring substances could have affected overall toxicity in some studies; however members had methodological concerns in these studies. Members were aware that some flavouring substances used in E(N)NDS may have been assessed for potential mutagenicity by authoritative bodies in relation to food. It was unclear whether the evaluation of potential mutagenicity of flavouring substances for food use would be relevant to inhalation exposure from the use of E(N)NDS.

For the non-standard studies (i.e. not conducted to OECD Test Guidelines) as a whole, the COM considered that there was no consistency in the assessment of mutagenicity or exposure, which made it difficult to evaluate the potential mutagenicity of E(N)NDS. However, members did not identify any mutation specific to E(N)NDS that are not produced by tobacco products.

In conclusion, members considered that although there was a breadth of evidence reported, 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 did not show
consistency and had not been repeated by other investigators. Members also
expressed concern that some studies reported genotoxicity only when wider
toxic effects were also observed. It was possible to conclude that this limited
evidence base did not indicate any specific mutagenic risks from E(N)NDS that
were not observed with conventional cigarette products. However, 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.
COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper for information on COM and COC consideration of genotoxicity and carcinogenicity risks

Discussion paper CC/2018/01 and associated minutes.

PHE COT Secretariat
November 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 (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.

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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 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

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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-naphthylamine (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, had 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 m³ 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°C and 300°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, surface area, PM10, vaping patterns and e-cigarette consumption.

19. In mainstream EN(N)DS aerosol, the authors reported higher average particle numbers (2.34 ± 0.5 ×10⁸ and 2.23 ± 0.8 and part. cm⁻³ with and without nicotine, respectively at 37°C) when compared with mainstream smoke of CC (data for comparison taken from published studies). At the higher temperature (300°C) particle numbers were lower, both with and without nicotine (7.02 ± 0.8 and 6.23 ± 0.5 x 10⁷ part.cm⁻³ respectively), than in mainstream EN(N)DS aerosols at 37°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°C with and without nicotine (9.08 ± 0.2 and 6.30 ± 1.3 x 10³ part.cm⁻³ respectively) and at 300°C with and without nicotine (8.92 ± 0.2 and 5.97 ± 1.3 x 10³ 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 10¹¹ nm² cm⁻³, with and without nicotine respectively) at 37°C when compared with mainstream smoke of CC (data for comparison taken from published studies). At the higher temperature (300°C) the surface area of particles in the EN(N)DS aerosol were lower those at 37°C, both with and without nicotine (3.35 ± 1.5 and 2.48 ± 0.8 x 10¹⁰ nm² 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°C, with and without nicotine (5.90 ± 1.4 and 5.16 ± 0.8 x 10⁷ nm² cm⁻³ respectively) and 300°C with and without nicotine (5.32 ± 1.4 and 3.51 ± 0.8 x 10⁷ nm² 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

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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 $5.42 \times 10^1$ mm$^2$ puff$^{-1}$ for CC and EN(N)DS, respectively; for females: $4.5 \times 10^2 - 9.3 \times 10^2$ and $4.93 \times 10^1$ 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 $2.4 \times 10^0$ mg puff$^{-1}$ for CC and EN(N)DS, respectively; for females: $3.4 \times 10^2 - 5.6 \times 10^2$ and $2.17 \times 10^0$ 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 $7.26 \times 10^{-6}$ and $7.3 \times 10^{-6}$ respectively for males, and $6.28 \times 10^{-6}$ and $6.11 \times 10^{-6}$ 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 $2 - 6 \times 10^{-4}$ related to CC use.

27. For second-hand CC and EN(N)DS aerosol, ECLR values with and without nicotine were $2.7 \times 10^{-8}$ and $1.29 \times 10^{-8}$ in males and $2.62 \times 10^{-8}$ and $1.24 \times 10^{-8}$ 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 $1 \times 10^{-5}$ 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 \textit{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 $4 \times 10^{-1}$ to around $7 \times 10^{-6}$. 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 $1.8 \times 10^{-3}$. 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) $\geq$ nicotine inhaler; $2.4 \times 10^{-2}$, $5.7 \times 10^{-4}$, $9.5 \times 10^{-5}$ and $8.9 \times 10^{-6}$ 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.

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$^4$ 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 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.

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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?
Abbreviations/Glossary

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


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**
  

- 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
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.


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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

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.