TOX/2018/46

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

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 7: Additional information on developmental toxicity studies of E(N)NDS aerosols.

1 Background

1. The COT is reviewing the potential toxicity of electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (collectively abbreviated to E(N)NDS). As part of this review, at the July 2018 COT meeting a paper reviewing studies that have evaluated the potential toxicity of E(N)NDS aerosols was discussed (TOX/2018/24). Members requested further information on the studies relating to potential developmental toxicity that could occur in offspring as a result of maternal exposure to E(N)NDS aerosols. This paper provides more information on these studies, plus an update of subsequently published literature relating to this topic.

2 Introduction

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

3. Constituents that have been identified in E(N)NDS liquids and/or aerosols include propylene glycol (PG), vegetable glycerine (VG, glycerol), water, nicotine, carbonyls, volatile organic compound (VOCs), tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), metals, ethanol, ethylene glycol, di-ethylene glycol, flavouring compounds, flavour enhancers, sweeteners, and phenolics. Data on reported levels of some of these constituents were summarised in discussion paper, TOX/2018/16, presented at the February 2018 COT meeting.

3 Literature searches and scope of the review

4. Five publications reporting studies of developmental toxicity following exposure to E(N)NDS aerosols were identified by literature searches to 06/04/2018, as described in TOX/2018/24. An updated search was carried out on 23/10/2018, which identified two additional publications.

4 Studies in humans

5. No reports describing studies that assessed the effects of exposure to E(N)NDS products during pregnancy or lactation in humans were found.

6. A description of one randomised clinical trial (RCT) currently in preparation was identified. This study is being led by investigators at Queen Mary University of London. The primary and secondary endpoints of the trial are listed, respectively, as: “To assess long-term (end of pregnancy) efficacy of electronic cigarettes (EC) compared to efficacy of nicotine transdermal patches when used to help pregnant smokers stop smoking”, and “To assess safety of electronic cigarettes (EC) compared to safety of nicotine transdermal patches when used to help pregnant smokers stop smoking; to assess effects of the two interventions on changes in smoke intake and in nicotine intake, effects on short-term abstinence; and adherence to each treatment”. Details of the proposed study are available at: https://www.journalslibrary.nihr.ac.uk/programmes/hta/155785/%23/# (accessed 14/09/18).

5 Studies in animal models

7. A small number of studies have been reported that have investigated effects of exposure to E(N)NDS aerosols during gestation and/or the early postnatal period in mice. Potential adverse effects on development of respiratory, nervous, and metabolic systems have been observed in association with nicotine and/or non-nicotine constituents of the aerosols. These studies have generally reported exposures to individual E(N)NDS constituents only in terms of concentration in the E(N)NDS liquid used to produce the aerosol, but not the actual levels to which the test animals were exposed via aerosol. A summary of data reported is given in the following paragraphs.

5.1 Neurodevelopment

8. A study was carried out to assess the effects of exposure during gestation and lactation to E(N)NDS aerosol, both with and without nicotine, on gene expression markers of neurodevelopment. Pregnant C57BL/6J mice were whole-body exposed to filtered room air (control) or E(N)NDS aerosol[1] (blu, classic tobacco flavour), with or without nicotine (13–16 mg/mL)[2], for 3 hours per day, 5 days per week, throughout gestation. Average particulate concentrations in the exposure chambers were 25.6 mg/m^3 and 30.7 mg/m^3 total suspended particulates, with and without nicotine, respectively. Pups received the same exposure from PND4–PND6 through lactation to 1 month of age, at which point brain frontal cortex tissue was subjected to transcriptomic analysis. There were no effects of E(N)NDS aerosols with or without nicotine on birth weight or pup weight gain compared with controls (Lauterstein et al. 2016).

9. Results of transcriptomic analyses were stratified by gender and exposure (with/without nicotine). Alterations in gene expression in the frontal cortex were detected in both groups (with/without nicotine) compared with controls. Compared with air-exposed controls, in the groups exposed to the E(N)NDS aerosol, the numbers of genes showing significantly altered expression were: female/without nicotine (2630); female/with nicotine (1393); male/without nicotine (2615); male/with nicotine (152). Overall, 109 genes were commonly altered in all 4 groups. Looking at the top 5 predicted disease and disorder categories for each exposure group, Ingenuity Pathway Analysis (IPA) indicated predicted changes in disease and disorder outcomes including cancer, organismal injury and abnormalities, gastrointestinal disease, neurological disease, and psychiatric disorders in all groups except the male/with nicotine group, in which cancer, organismal injury and abnormalities, gastrointestinal disease, dermatological diseases and conditions, and connective tissue disorders were identified. The predominant predicted disease and biological functions included decreases in memory, cognition, learning and neurotransmission, and increases in hyperactive behaviour, emotional behaviour and organismal death (all groups except male/with nicotine); decreases in dendritic growth/branching and quantity of neurons, increases in seizure disorder and seizures in all treatment groups; and decrease in locomotion (male/with nicotine group). qPCR analysis was performed for 7 genes of interest, *Ngfr*, *Chat*, *Bdnf*, *Gdnf*, *Gal*, *Tbr1*, and *Adra1d*, and results were reported to be similar to those for RNA-Seq analysis (Lauterstein et al. 2016).

10. The authors concluded that similar gene expression changes were observed in female offspring exposed to E(N)NDS aerosol with and without nicotine as those seen in male offspring exposed to aerosol without nicotine. In contrast, male offspring exposed to aerosol with nicotine exhibited a smaller number of gene expression changes as well as different IPA-predicted diseases and disorders. The authors proposed that a possible explanation for this difference could be the

[1] Aerosol described as 73% PG and/or VG, 15% water, 11% flavourings, 1% nicotine
[2] 35 mL puff volume, 4-s puffs, 30-s intervals, mixed with filtered air.

inhibitory action of nicotine on aromatase enzyme (resulting in oestrogen deficiency), and they noted that the timing of euthanasia, which was performed at the beginning of puberty in this study, may be relevant to this hypothesis. The authors were surprised to find that exposure to aerosols without nicotine produced the greatest number of significant gene-expression changes in offspring, and commented that this may suggest that aerosol components other than nicotine may have been involved (Lauterstein et al. 2016).

11. A follow-on paper from the set of studies described by Lauterstein et al. (2016) reported parameters of neuroinflammation in the frontal cortex and hippocampus, neurotrophin gene expression in the hippocampus, and serum cytokine levels from the same experimental setup. A re-analysis of IPA data for the frontal cortex was also carried out. Experimental details (animals, exposures) were as reported by Lauterstein et al. (2016) (paragraph 8). Measured aerosol exposure concentrations were 25.6 mg/m^3 (with nicotine) and 30.7 mg/m^3 (without nicotine) total particulates (Zelikoff et al. 2018).

12. Immunostaining for markers of neuroinflammation (glial fibrillary acidic protein (GFAP) for astrocytes, ionized calcium-binding adaptor molecule 1 (Iba-1) for microglia) showed significantly increased Iba-1 in the cornis ammonis (CA) 1 region of the hippocampus in male and female mice exposed to aerosol without nicotine, but not in the groups exposed to E(N)NDS aerosol with nicotine. There was a nonsignificant tendency for the same pattern of effects in the frontal cortex. No changes in Iba-1 were observed in the hippocampus regions CA3 or dentate gyrus. There were no changes in GFAP in any exposure groups. The authors noted that exposure to aerosol without nicotine was more inflammatory than exposure to aerosol with nicotine, and suggested possible hypotheses for this including: 1. a protective effect of nicotine via promotion of neurogenesis; 2. a speculative effect of PG to increase inflammation (Zelikoff et al. 2018).

13. Expression of neurotrophin genes, *Ngfr*, *Bdnf*, and *Gdnf* was evaluated in the hippocampus. *Ngfr* and *Bdnf* were significantly downregulated in both aerosol groups, with or without nicotine (sexes pooled) compared with controls. No changes were observed for *Gdnf* expression. Serum levels were measured for the cytokines, interleukin (IL)-6, tumour necrosis factor alpha (TNFα), IL1β, interferon gamma (IFNɣ), IL-2, and monocyte chemoattractant protein-1 (MCP-1). IL1β was significantly reduced in the groups (male and female) exposed to aerosol with and without nicotine. IL-2 was significantly reduced in the aerosol with nicotine group, with a nonsignificant trend to the same finding for exposure to aerosol without nicotine. There was also a nonsignificant trend to reduction in IL-6 level in mice exposed to aerosol with and without nicotine. There were no changes in TNFα, IFNɣ, or MCP-1 levels. The authors suggested that the similar effects of aerosol with and without nicotine on neurotrophin gene expression and serum cytokine levels indicated that E(N)NDS constituents other than nicotine could be responsible for these effects (Zelikoff et al. 2018).

14. The re-evaluation of frontal cortex IPA indicated the following numbers of genes overlapping with the IPA 'affects inflammation of the CNS' molecule list: aerosol without nicotine/males (27); aerosol without nicotine/females (24); aerosol with nicotine/males (6); aerosol with nicotine/females (13) (Zelikoff et al. 2018).

15. Overall, the authors concluded that exposure to E(N)NDS aerosols, both with or without nicotine, poses a risk to the developing CNS (Zelikoff et al. 2018).

16. Another study in mice indicated effects of exposure to E(N)NDS aerosol containing nicotine during late gestation and early postnatal life (correlating to the 3rd trimester of human pregnancy) on subsequent behavioural parameters. Pregnant C57BL/6J mice were whole-body exposed to PG or PG/2.4% nicotine aerosol from GD15 to GD19, and mothers and offspring were exposed from PND2 to PND16. Exposures were achieved as 6-s puffs every 15 s into a 13.5 x 9 x 8.7 cm chamber, from a total of 600 µL liquid, over a period of approximately 20 min, once per day. Exposure concentrations were not reported. Mean pup weight at birth and throughout postnatal development was significantly lower in the PG group without nicotine compared with the control or the group exposed to PG with nicotine. Mean pup weight in the group exposed to PG with nicotine was significantly lower than that of controls from PND7 onwards. Pups in the PG with nicotine group (postnatally) were estimated to be exposed to 2.1 mg/day, excluding exposures from additional sources such as milk and fur. Mean serum cotinine levels, measured at PND14 in female offspring, were 23.7 ng/mL in the group exposed to PG with nicotine; 2.8 ng/mL in the group exposed to PG without nicotine; and 1.0 ng/mL in controls. The authors noted that similar levels have been reported in newborns of mothers who smoked. One study reported approximately 32 ng/mL to 59 ng/mL cotinine in plasma of newborns, born to mothers who were moderate or heavy CC smokers, respectively, measured 48 hours after birth (Ivorra et al., 2014, *cited in* Smith et al. (2015)). Another study reported a mean plasma cotinine concentration of 76 ng/mL in newborns of mothers who were reported as being smokers (Chazeron et al., 2008, *cited in* Smith et al. (2015)) (Smith et al. 2015).

17. In the above mentioned study, behavioural tests to assess sensorimotor, affective, and cognitive domains were performed on adult male offspring at 14 weeks postnatally. Behavioural alterations were observed in offspring exposed to PG with nicotine compared to controls or offspring exposed to PG without nicotine. Authors noted this indicates increased locomotor activity (significant increase in mean number of rearing activity in open field test and head dips in zero maze test), and tendency towards increased cognitive flexibility (significantly more than 25% of time in the new location in water maze test). The authors concluded that the findings indicated that nicotine exposure from E(N)NDS may cause persistent behavioural changes when exposure occurs during a period of rapid brain growth (Smith et al. 2015).

18. A group of researchers based at the University of Sydney have carried out studies of the effects of exposure to E(N)NDS aerosol, with and without nicotine,

during gestation and lactation in mice (Chen et al. 2018a, Chen et al. 2018b, Nguyen et al. 2018) (see also paragraphs 27–32, 33–36, and 37–44). One report from this set of studies described assessment of effects on brain development and behaviour (Nguyen et al. 2018). Adult female Balb/c mice (n = 8 per group) were exposed to E(N)NDS aerosol (50/50 PG/VG, tobacco flavour), either with [Ecig(+nic)] or without [Ecig(–nic)] 18 mg/mL nicotine, or ambient air (sham), in a 9 L exposure chamber, for 15 min twice per day with a 5-min interval, from 6 weeks before mating until pups were weaned at PND20. Measured aerosol exposure concentrations were not reported, nor mouse cotinine levels (Nguyen et al. 2018).

19. Behavioural assessments were carried out on adult male offspring at 12 weeks postnatally. Compared with the sham group, the [Ecig(+nic)] but not [Ecig(–nic)] offspring showed indicators of short-term memory deficit (assessed by novel object recognition test). Both [Ecig(–nic)] and [Ecig(+nic)] aerosol exposures were associated with indicators of increased anxiety in some aspects of the elevated plus maze (EPM) test (significantly increased time in open arms of maze by [Ecig(–nic)] and [Ecig(+nic)] groups; significantly increased number of head dips in the [Ecig(+nic)] group; significantly increased unprotected stretches in the [Ecig(–nic)] group). Both [Ecig(–nic)] and [Ecig(+nic)] showed significantly increased locomotor activity (number of centre crosses in the EPM test) compared with the sham control group (Nguyen et al. 2018).

20. Tissues were collected from male offspring euthanized at PND1, PND20, or 13 weeks postnatally. Global DNA methylation was significantly increased in whole brain of [Ecig(–nic)] but not [Ecig(+nic)] offspring compared with the sham group, evaluated at PND1 and PND20. Evaluation of global DNA methylation was performed specifically for the hippocampus at 13 weeks postnatally, with no significant differences between the 3 groups. Detailed gene expression analyses were performed, showing various changes in genes associated with the modulation of neurological activity. The authors suggested that the changes in global methylation at PND1 and PND20 (in the Ecig(-nic) group only) might be related to substances transferred via the breastmilk (Nguyen et al. 2018).

21. The authors concluded that the memory deficits seen in this study were due to intrauterine nicotine exposure, and that changes in other behaviours seen in EPM tests may be due to other constituents within E(N)NDS aerosols (Nguyen et al. 2018).

5.2 Respiratory system development

22. McGrath-Morrow et al. (2015) found that nicotine exposure from E(N)NDS aerosol affected alveolar growth and lung cell proliferation. Neonatal C57BL/6J mice were whole-body exposed to aerosol produced from 400 µL E(N)NDS liquid (PG or PG/1.8% nicotine; no flavouring) from PND1 to PND10, once per day on PND1 and PND2, then twice per day. Exposure was achieved from 6-s puffs every 15 s over approximately 20 min. Exposure concentrations were not reported. Control mice were kept in room air. Results were reported for ‘Trial 1’ and ‘Trial 2’, although the

report does not describe what Trial 1 and Trial 2 were (McGrath-Morrow et al., 2015).

23. Plasma and urinary cotinine levels in offspring were measured at PND10, reported for Trial 1. Mean levels in plasma and urine, respectively, were: < 5 ng/mL and < 10 ng/mL for both control and PG/no-nicotine groups; 63.3 ng/mL and 892.5 ng/mL for the PG/nicotine group. As compared with controls, total body weight at PND10 was significantly decreased (Trial1 and Trial 2) in both the PG/no-nicotine (11.5% decrease) and PG/nicotine (13.3% decrease) groups. Body weight reduction in PG/nicotine offspring was significantly correlated with plasma cotinine level (McGrath-Morrow et al. 2015).

24. Mean linear intercept (MLI), a measure of mean free distance into airspaces, taken as a marker of postnatal alveolar growth, was measured in offspring at PND10. The MLIs were reported as higher in offspring exposed to PG/nicotine compared with controls in Trial 1 ($p < 0.054$) and Trial 2 ($p < 0.006$), but were not associated with cotinine levels. The MLI was not significantly different between PG/no-nicotine and control offspring (Trial 1, $p = 0.27$; Trial 2, $p = 0.79$). The MLI was significantly higher in PG/nicotine offspring than PG/no-nicotine in Trial 2 ($p < 0.014$) but not in Trial 1 ($p < 0.34$) (McGrath-Morrow et al. 2015).

25. Alveolar cell proliferation was evaluated by immunohistochemical staining for the marker, KI67. Staining was significantly decreased in offspring exposed to PG/nicotine compared with controls and compared with those exposed to PG/no-nicotine. There were no differences in levels of markers of apoptosis or oxidative stress (McGrath-Morrow et al. 2015).

26. The authors concluded that the findings from this study indicated that exposure to nicotine-containing E(N)NDS can cause systemic absorption of nicotine and modest impairment of lung growth during early postnatal life. The authors also commented that exposure to aerosol without nicotine caused decreased body weight gain compared with room air-exposed controls, suggesting that PG exposure alone may disrupt postnatal growth, although it was noted that others factors may also need to be taken into consideration, such as stress/altered feeding behaviours related to the different treatment protocol of controls compared with aerosol exposure groups (McGrath-Morrow et al. 2015).

27. Chen et al. (2018a) reported that maternal exposure to E(N)NDS aerosol, with or without nicotine, altered cytokine levels in both maternal and offspring lungs. Adult female Balb/c mice were exposed to E(N)NDS aerosol (50/50 PG/VG, tobacco flavour), either with ('E-cig18') or without ('E-cig0') 18 mg/mL nicotine[3], for 15 min, twice per day with a 5-min interval, from 6 weeks before mating until pups were weaned at PND20. To achieve this, the adult females were removed from the home cage and placed in a 9 L exposure chamber. Adult male mice and offspring did not undergo exposures, but remained in the home cage. Measured exposure

[3] Aerosol generated by KangerTech NEBOX, 4 x 5-s puffs at 30 W, 20-s interval

concentrations were not reported. Control animals underwent the same procedure, but were exposed to room air instead of E(N)NDS aerosol. Male offspring were studied at PND1, PND20, and at 13 weeks postnatally. Mothers were studied when pups were weaned.

28. Mean blood cotinine levels in offspring at weaning were 9.12 ng/mL (E-cig18), 3.31 ng/mL (E-cig0), and 2.83 ng/mL (control) (Chen et al. 2018a).

29. After the first 6 weeks of exposure, measurement of maternal lung proinflammatory cytokine levels showed increased IL1-β in E-cig18, increased IL-6 in E-cig0, and increased TNF-α in both E-cig0 and E-cig18, compared with controls (Chen et al. 2018a).

30. Proinflammatory cytokines were measured in adult offspring lung at 13 weeks postnatally. Compared with controls, IL1-β was significantly decreased in both E-cig0 and E-cig18, TNF-α was significantly increased in E-cig18 only, and IL-6 was increased non-significantly ($p = 0.05$) in E-cig0 only. These changes were correlated with changes in signalling pathway protein expression levels (Chen et al. 2018a).

31. Gene expression markers of alveolar development were studied in PND1 and PND20 offspring lungs. Increased expression of *PDGF* mRNA was noted at PND20, but not PND1, in E-cig18 and E-cig0 offspring, compared with controls. There were no differences between groups in ephrine B2 or surfactant protein C (*Sftpc*) mRNA levels at either PND1 or PND20. Global DNA methylation in the lungs was increased 3-fold in E-cig0 and 2-fold in E-cig18 offspring at PND1, compared with controls (Chen et al. 2018a).

32. The authors concluded that both nicotine and non-nicotine constituents induce inflammatory responses in the lungs of both mothers and offspring, that different signalling pathways may be altered in mothers and offspring, and that epigenetic modifications are likely to contribute in the offspring (Chen et al. 2018a).

5.3 Effects on body weight, body fat, and energy homeostasis in offspring

33. As described in paragraph 27-28, in the study reported by Chen et al. (2018a), adult female Balb/c mice were exposed to E(N)NDS aerosol (50/50 PG/VG, tobacco flavour), either with ('E-cig18') or without ('E-cig0') 18 mg/mL nicotine[4], for 15 min, twice per day with a 5-min interval, from 6 weeks before mating until pups were weaned at PND20. Control animals underwent the same procedure, but were exposed to room air instead of E(N)NDS aerosol.

34. After the first 6 weeks of exposure, E-cig0 mothers had only gained one-third of the weight of control mice, while weight gain in E-cig18 mice was not affected. There were no differences in weight gain or liver weight between maternal groups at the end of the experiment, but retroperitoneal fat mass was significantly reduced in

[4] Aerosol generated by KangerTech NEBOX, 4 x 5-s puffs at 30 W, 20-s interval

both groups of aerosol-exposed mothers compared with controls (Chen et al. 2018a).

35. In offspring, body weight did not differ between the 3 groups at PND1. At PND20, E-cig0 offspring were significantly heavier and E-cig18 were significantly lighter than controls. Liver weight, as a percentage of body weight, was significantly increased in E-cig18 offspring but not E-cig0 offspring compared with controls. E-cig0 and E-cig18 offspring had significantly increased retroperitoneal fat mass compared with controls (E-cig0 > E-cig18). Epididymal fat mass was significantly increased in E-cig0 compared with both E-cig18 and controls. At 13 weeks postnatally, body weights did not differ between groups. Liver weight was reduced in E-cig0 compared with the other 2 groups. Retroperitoneal fat mass was increased in both E-cig0 and E-cig18 compared with controls (Chen et al. 2018a).

36. Mean blood cotinine levels in offspring at weaning were 9.12 ng/mL (E-cig18), 3.31 ng/mL (E-cig0), and 2.83 ng/mL (control) (Chen et al. 2018a). Data relating to respiratory outcomes in this study are described in paragraphs 29–32.

37. A follow-on study from those of Chen et al. (2018a) (paragraphs 27–32 and 33–36) and Nguyen et al. (2018) (paragraphs 18–21) compared effects on energy homeostasis of maternal exposures during pregnancy and lactation to cigarette smoke extract and E(N)NDS aerosol, with or without nicotine, on offspring in mice (Chen et al. 2018b).

38. Adult female Balb/c mice (n = 8 per groups) were exposed to E(N)NDS aerosol (50/50 PG/VG, tobacco flavour, Vaper Empire, Australia) either with [E-cig18] or without [E-cig0] 18 mg/mL nicotine[5], smoke extract [SE][6], or ambient air [Sham]. Exposures were carried out in a chamber, for 30 min, twice per day, from 6 weeks before mating until pups were weaned at PND20. The adult male mice and offspring were not exposed. Measured aerosol exposure concentrations were not reported, however nicotine dosage per treatment was described as “equivalent to 2 cigarettes (2.4 mg)”. Two additional subgroups were created from the [SE] group: replacement of SE with E-cig18 vapour from mating until weaning [Replacement]; supplementation of SE with L-carnitine[7] during gestation and lactation [SE + LC] (Chen et al. 2018b).

39. Male offspring (1 per litter) were euthanized at weaning (PND20). Body weight and epidydimal fat mass were recorded, and total mRNA extracted from whole brain was analysed for expression of the genes encoding the regulators of appetite and food intake, neuropeptide Y (NPY), NPY Y1 receptor (Y1R), pro-opiomelanocortin (POMC), melanocortin receptor (MC4R), single-minded gene (Sim) 1, and leptin receptor (Ob-Rb), and also the oxidative stress marker, inducible nitric oxide synthase (iNOS). Analysis of expression levels of proteins was not undertaken due

[5] Aerosol generated by KangerTech NEBOX, 4 x 5-s puffs at 30 W, 20-s interval
[6] 2 cigarettes (Winfield Red, VIC, Australia)
[7] An antioxidant that has shown benefits on ‘offspring brain health’ when supplemented to pregnant mice exposed to SE.

to insufficient sample availability. For all measured parameters, 2 sets of comparisons were made: 1] [Sham], [e-cig0], and [e-cig18]; 2] [Sham], [SE], [Replacement], and [SE + LC] (Chen et al. 2018b).

40. At PND20, [E-cig0] offspring had significantly higher body weight, fat mass, and % fat compared with [E-cig18] and with [Sham], while values for [E-cig18] were not significantly different from [Sham] (Chen et al. 2018b).

41. There were no significant differences in body weight, fat mass, or % fat between [Sham] and either [SE] or [SE + LC]. However, in [Replacement] offspring, fat mass and % fat were significantly reduced compared with [SE] (Chen et al. 2018b).

42. For gene expression analyses, NPY and iNOS were significantly higher in [E-cig0] compared with [Sham] and with [E-cig18], and MC4R and Ob-Rb were significantly higher in [E-cig0] compared with [E-cig18]. There were no significant differences between [E-cig18] and [Sham] (Chen et al. 2018b).

43. Exposure to [SE] was associated with significantly increased expression of NPY, NPY Y1R, and iNOS compared with [Sham], [Replacement], and [SE + LC], and also with increased expression of MC4R and Ob-Rb compared with [Sham]. In [Replacement] offspring, NPY, NPY Y1R, MC4R, Sim, Ob-Rb, and iNOX were significantly lower compared with [SE], and NPY, MC4R, and Ob-Rb were significantly lower compared with [SE + LC], with the authors noting that “the effect of Replacement to normalise brain markers was more potent than maternal L-carnitine supplementation.” (Chen et al. 2018b).

44. The authors commented that this study showed that nicotine-free E(N)NDS aerosol induces significant adiposity, dysregulation of brain metabolic regulatory pathways and increase iNOS expression, and concluded that nicotine-free may be more harmful than nicotine-containing E(N)NDS aerosol if the same amount is inhaled. They also commented on the fact that replacement of CC smoke with nicotine-containing aerosol normalised the effects of CC smoke on the regulatory pathways evaluated, supporting an assumption that E(N)NDS vapour may be safer than smoking CC, and highlighting the potential role of CC components other than nicotine in maternal programming of metabolic regulation in offspring (Chen et al. 2018b).

6 Summary

45. Searches were carried out to identify publications describing studies of developmental toxicity to offspring of exposure to E(N)NDS aerosols, with relevance to exposures that would occur in humans via maternal exposure to E(N)NDS.

46. No data were found on the effects of maternal exposure to E(N)NDS aerosols on offspring development in humans. A description of one RCT in preparation was identified. This study, which is being led by investigators at Queen Mary University of

London, will aim to assess efficacy and safety of ENDS compared with transdermal nicotine replacement therapy (NRT) patches to help pregnant smokers stop smoking (https://www.journalslibrary.nihr.ac.uk/programmes/hta/155785/%23/#/, accessed 14/09/18).

47. A total of 7 publications, from 3 different research groups, were identified that describe studies of the effects on offspring development of exposures of mice to E(N)NDS aerosols *in utero*, via breast milk, and/or by direct exposure in the early postnatal period. These studies reported adverse effects of nicotine-containing and nicotine-free aerosols on development of the respiratory, neurologic, and metabolic systems.

48. A set of studies evaluated neurodevelopmental effects in mouse offspring of maternal exposures throughout gestation and lactation to E(N)NDS aerosols produced from a tobacco-flavoured 'blu' E(N)NDS liquid containing 0 mg/mL or 13–16 mg/mL nicotine. Brain tissues were analysed at 1 month postnatally for markers of neuroinflammation and gene expression. Effects in both exposure groups were noted that were related to nicotine and/or other aerosol components, and in some cases were sex specific, with the authors concluding that exposure to E(N)NDS aerosols, with or without nicotine, poses a risk to the developing CNS (Lauterstein et al. 2016, Zelikoff et al. 2018). A study in which mice were exposed during gestation and lactation to E(N)NDS aerosols produced from a tobacco-flavoured e-liquid (50/50 PG/VG) containing 0 mg/mL or 18 mg/mL nicotine showed short-term memory deficits associated with exposure to nicotine-containing aerosol and behavioural alterations that were associated with exposures to aerosols with and without nicotine, in male offspring tested at adulthood. This led the authors to conclude that memory deficits were due to nicotine exposure while behavioural alterations may be due to other aerosol constituents (Nguyen et al. 2018). Finally, a study in which mice were exposed to E(N)NDS aerosol (PG or PG/2.4% nicotine) during late gestation and early postnatal life indicated that exposure to aerosol containing nicotine, but not without nicotine, led to altered behavioural test outcomes in adult male offspring (Smith et al. 2015).

49. Direct exposure of mouse pups during early postnatal life to E(N)NDS aerosol produced from an unflavoured E(N)NDS liquid containing PG/1.8% nicotine was associated with modest impairment of lung growth, while these effects were not observed with exposure to aerosol produced from a PG/0% nicotine E(N)NDS liquid (McGrath-Morrow et al. 2015). A separate study in which mice were exposed during gestation and lactation to E(N)NDS aerosols produced from a tobacco-flavoured E(N)NDS liquid (50/50 PG/VG) containing 0 mg/mL or 18 mg/mL nicotine revealed indicators of inflammatory responses in offspring lungs in both exposure groups, with some differences in specific findings between the two groups, leading the authors to conclude that both nicotine and non-nicotine components induce inflammatory responses. Global DNA methylation was increased in offspring lungs at birth in both groups (Chen et al. 2018a).

50. A follow-on study from the set of studies by Chen et al. (2018a) and Nguyen et al. (2018), in which mice were exposed during gestation and lactation to E(N)NDS aerosols produced from a tobacco-flavoured e-liquid (50/50 PG/VG) containing 0 mg/mL or 18 mg/mL nicotine also compared effects on energy homeostasis, in this case also including comparisons with groups of mice exposed to tobacco smoke extract. Nicotine-free E(N)NDS aerosol was associated with increased weight gain and adiposity, and dysregulation of brain metabolic regulatory pathways compared with the other exposures, leading the authors to conclude that that nicotine-free may be more harmful than nicotine-containing E(N)NDS aerosol if the same amount is inhaled (Chen et al. 2018b). Conversely the studies of Smith et al. (2015) and McGrath-Morrow et al. (2015) had both reported effects of reduced offspring body weight associated with exposures to aerosols of PG and PG/nicotine, while in the study of Lauterstein et al. (2016) the E(N)NDS aerosol exposures with and without nicotine had no effect on offspring body weight.

7 Questions for the Committee

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

i. Is the Committee able to draw any conclusions from the data presented on the risks to development in the offspring of maternal exposure to E(N)NDS aerosols or risks relative to smoking conventional cigarettes?

ii. Are there any particular aspects of this paper that should be captured when a COT statement on E(N)NDS is prepared?

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

CA	Cornis ammonis
CC	Conventional cigarette
CNS	Central nervous system
EC	Electronic cigarette
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
EPM	Elevated plus maze
GD	Gestation day
GFAP	Glial fibrillary acidic protein
Iba-1	Ionized calcium-binding adaptor molecule 1
iNOS	Inducible nitric oxide synthase
IPA	Ingenuity Pathway Analysis
LC	L-carnitine
MC4R	melanocortin receptor
MCP-1	Monocyte chemoattractant protein-1
MLI	Mean linear intercept
NPY	neuropeptide Y
NPY Y1R	NPY Y1 receptor
NRT	Nicotine replacement therapy
Ob-Rb	Leptin receptor
OR	Odds ratio
PAH	Polycyclic aromatic hydrocarbon
PG	Propylene glycol
PND	Postnatal day
POMC	pro-opiomelanocortin
RCT	Randomised clinical trial
SE	Smoke extract
Sim 1	Single-minded gene
TSNA	Tobacco-specific nitrosamine
VG	Vegetable glycerine (glycerol)
VOC	Volatile organic compound

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