TOX/2019/01

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 8: Additional information on toxicity in adolescent and young adult users.

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 evaluated the potential toxicity of E(N)NDS aerosols was discussed (TOX/2018/24) and an overview of three published reviews of nicotine toxicity was also presented (TOX/2018/25). Members requested further information on the studies relating to developmental toxicity that could occur in offspring as a result of parental exposure to E(N)NDS aerosols and, specifically, to nicotine. These areas were addressed by papers TOX/2018/46 (E(N)NDS aerosols) and TOX/2018/45 (nicotine) discussed at the December 2018 COT meeting. Further to these discussions, Members requested a summary of information on the potential toxicity of exposure to E(N)NDS aerosols during adolescence. This paper provides more information on this topic, including a recap of relevant literature previously described in TOX/2018/24 and TOX/2018/25 and an update of recent literature relating to toxicity of exposure to E(N)NDS or nicotine in adolescence and young adulthood.

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 in E(N)NDS liquids and aerosols were summarised in discussion paper, TOX/2018/16, presented at the March 2018 COT meeting.

Literature searches and scope of the review

4. Literature relating to toxicity of nicotine and of E(N)NDS aerosols in adolescents and young adults was identified from:

A. Relevant data that have been presented in previous COT discussion papers on E(N)NDS.

B. Updated literature searches relating to toxicity in adolescents and young adults of E(N)NDS (01/04/2018–11/12/2018) and nicotine (01/01/2016–11/12/2018), as described in Annex A.

C. Any further literature of particular relevance highlighted in the text/reference lists of publications identified in B, above.

5. The text below is an overview of the dataset identified. Individual studies are not described in detail. Where text from previous COT discussion papers or published review articles are referred to, the full text of this review literature is reproduced in Annexes B, C and D.

Nicotine

Literature reviewed in previous COT discussion papers

6. Paragraph 15 of COT discussion paper TOX/2018/25 briefly summarised the effects of nicotine in youth users, overviewing the section on this aspect in the 2016 U.S. Surgeon General Report ‘E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General’ (pages 104–107 of Chapter 3 ‘Health Effects of E-cigarette Use’) (U.S. Department of Health and Human Services 2016). An expanded version of the summary from TOX/2018/25 is given in paragraphs 7–11 below, and the full text of pages 104–107 from Chapter 3 of the U.S. Surgeon

General report, where relevant literature citations can be found, is appended at Annex B.

7. The report by the Surgeon General notes that there is substantial evidence that nicotine can negatively affect adolescent brain development. Although limited epidemiological data are available, animal studies provide evidence of biological plausibility and the human and animal data, overall, provide evidence for neuroteratogenic and neurotoxic effects of nicotine on the developing adolescent brain.

8. Rodent models are described as being appropriate for studying the neurobiology of brain development in human teenagers. Expression and functional activity of acetylcholine receptors (AChRs) is higher in the adolescent forebrain compared with adult rodent forebrain. Studies have shown increased sensitivity to the rewarding effects of very low doses of nicotine in adolescent rats, as well as decreased levels of aversion/negative effects of short-term nicotine exposure. Based on this the Surgeon General highlights “the possibility that human adolescents might be particularly vulnerable to developing dependency to and continuing to use e-cigarettes”. It is also suggested that nicotine use by adolescents may lead to behaviours that have been clearly associated with CC smoking in adolescence, such as reward-seeking behaviours (‘gateway’ effects to use of other substances) and increased risk-taking. Studies in rodents have shown that nicotine exposure during adolescence increases the reinforcing effects of other drugs of abuse, including cocaine, methamphetamine, and alcohol. Some rodent studies have shown effects of very low doses of nicotine during early adolescence to produce long-lasting changes in D2 and D3 dopamine receptors and self-administration of other abused drugs. Such effects were not observed in late adolescence or adulthood. Nicotine exposure of adolescent rats induced long-lasting dendritic remodelling in a brain region critical to reward learning and addiction.

9. Some studies have suggested that CC smoking in adolescence is associated with both acute and long-term effects on attention and memory, and studies in rats have shown that adolescent nicotine exposure induces synaptic changes in prefrontal cortical regions responsible for attention, memory, and cognition. Treatment of adolescent, but not post-adolescent, rats with nicotine led to diminished attention span and enhanced impulsivity in adulthood, associated with altered regulation of prefrontal cortex excitatory synapse function. In another study, adolescent exposure of mice to nicotine led to altered hippocampal function (indicated by alterations in contextual fear conditioning, a memory-dependent process) in adulthood.

10. The potential association of CC smoking with mood disorders in adolescence is also discussed, both in terms of the use of nicotine to self-medicate, but also the possibility that nicotine exposure via CC smoking may contribute to the development of mood disorders. Epidemiological studies have indicated that tobacco use and tobacco dependence in adolescence are associated with increased risk of anxiety

disorders. Bidirectional relationships between adolescent CC smoking and disruptive disorders such as attention deficit hyperactivity disorder (ADHD), oppositional defiance disorder (ODD), and depression have been reported, with a suggestion of a causal relationship between teen smoking and onset of depression. Animal studies support the concept that adolescent nicotine exposure may lead to long-term changes in emotional responses, particularly anxiety and fear, and cause persistent alterations in serotonin systems involved in mediating mood disorders.

11. In summary, the narrative of the Surgeon General's report concludes that "*given the existing evidence from human and animal studies of the detrimental impact of nicotine exposure on adolescent brain development, the use of e-cigarettes by youth should be avoided and actively discouraged. Both preadolescence and adolescence are developmental periods associated with increased vulnerability to nicotine addiction, and exposure to nicotine during these periods may lead to long-lasting changes in behavioural and neuronal plasticity*." (U.S. Department of Health and Human Services 2016).

Additional literature

12. Searches were carried out to identify literature relating to toxicity and adverse effects of nicotine in adolescents and young adults published after the date-range covered by the searches carried out for the U.S. Surgeon General report described in paragraphs 7–11 above. A total of 335 citations were identified from searches of the Scopus and PubMed databases for the period 01/01/2016–11/12/2018, including review articles, data relating to human studies, and studies in animals.

13. Polosa et al. (2017) published a critique of the 2016 U.S. Surgeon General report described in paragraphs 7–11 above. In their critique of Chapter 3 on health effects, Polosa and colleagues comment that the evidence presented by the Surgeon General on potential harmful effects of nicotine exposure during adolescence (in humans) draws on existing literature from exposure to CC smoke, because no data are available for exposure to nicotine from ENDS aerosols. They highlight the possibility that this may lead to the risk of ENDS use by young people being portrayed as comparable to that of CC smoking. Polosa and colleagues also consider that the animal models described may be of limited relevance to real-world human ENDS use because of the dose and exposure regimens used. They also comment that literature presented by the Surgeon General on associations between CC smoking and cognitive, attention, and mood effects later in life is inconclusive, showing bidirectional associations that do not prove causality, and with likely confounders such as genetic predisposition and social influences.

14. England et al. (2017) published a review of the developmental toxicity of nicotine, focussing on effects on the respiratory and neurological systems, and covering exposure during pregnancy and during adolescence. Data from this review article relating to exposure during pregnancy were summarised in the previous COT discussion paper, TOX/2018/45. The section of the review article relating to adolescence is summarised in paragraphs 15–16 below. The full text of the

publication by England et al. (2017), in which relevant reference citations can be found, is appended at Annex C.

15. In the section covering adolescence, England et al. (2017) focus on effects on brain development, cognitive outcomes, and gateway effects to the use of other substances. It is noted that brain development continues well into the third decade of life and that adolescent and young adult brains differ from those of the fully mature adult, both physiologically and neurochemically. Synapses are more plastic, and adolescents have superior learning and memory skills compared with adults. However, this can be detrimental in the situation of inappropriate stimulation by neuroactive chemicals, for example activation and strengthening of reward circuits by addictive drugs. Human data relate to studies of CC smoking in human adolescents. The narrative notes that lifetime addiction to CC smoking is more likely in subjects who begin smoking in the teen years (compared with later), and symptoms of dependence develop at lower levels of exposure. This has been supported by studies of nicotine self-administration in adolescent and adult rodent models. Of particular concern is the prefrontal cortex, where circuit formation and neural remodelling continues into the 20s, including in dopamine (reward) and ACh (cognitive maturation) –associated systems. Studies have suggested that adolescent CC smokers show attenuated anticipation responses to non-drug (e.g. financial, food images) rewards compared with nonsmokers, including at low levels of CC smoking (≤ 5 cigarettes/day), with the implication that the use of extremely rewarding drugs such as nicotine leads to diminished perception of pleasure from non-drug rewards.

16. The review by England and colleagues notes that the small number of studies that are available suggest that CC smoking in adolescents is associated with adverse effects on cognition, with some gender differences, including effects on working memory and verbal memory, executive planning, auditory processing, and attentional network activity. Several mental health disorders that include changes in cognition are associated with higher rates of tobacco use, including schizophrenia, ADHD, depression, anxiety disorders, bipolar disorder, and others, and there is some evidence to support a bidirectional relationship. The involvement of nicotine is supported by findings from studies in rodents, which have shown that nicotine exposure during adolescence produces long-lasting deficits in learning and cognitive processes, including contextual processing, associated with changes to the hippocampus. These effects were not seen in nicotine-exposed adults. Rats exposed to nicotine during adolescence also had deficits in attention and displayed increased impulsivity in adulthood, which it is postulated may be related to effects on synaptic plasticity. Animal studies also support the concept of long-term adverse mental health effects associated with adolescent nicotine exposure, including increased anxiety, decreased sensitivity to natural rewards, and fostered depression-like behaviours. Changes in anxiety were associated with increased release of neuropeptides involved in stress responses in specific brain regions. In summary to this section on adolescent exposure to nicotine, England et al. (2017) commented that taken together, these findings raise serious concerns about the long-term impact of adolescent nicotine exposure on mental health through adverse effects on

cognition, anxiety, impulsivity, depression, and drug reward and reinforcement. In concluding the review article, the authors consider that, as the human brain does not reach full maturity until the mid-20s, it would be of benefit in terms of public health to restrict the sale of ENDS to persons of 21 years and older (England et al. 2017).

17. The updated literature search identified several further publications relating to nicotine and human adolescents or young adults. A substantial proportion of this literature comprised evaluations and commentaries on factors relating to E(N)NDS use and exposure (levels of use, expectations, opinions, social perspectives) in different populations of adolescents and young people. Some reports discussed the relationship of E(N)NDS use to subsequent initiation of CC smoking. Some studies had evaluated (multi)drug use (e.g. alcohol, nicotine, CC smoking, cannabis) in adolescents with psychiatric disorders or mental health problems, including ADHD, obsessive compulsive disorder (OCD), antisocial behaviour disorders, bipolar disorders, borderline and schizotypal personality types, dysthymia and depression, and sleep disorders. However, these studies evaluated drug use/screened levels in patients with diagnosed disorders or mental health issues (rather than prior to the appearance of symptoms) and generally did not focus solely on nicotine. Young people with ADHD were noted to have generally high levels of CC smoking. A small number of reports related to potential biological/health effects of nicotine, but these mostly addressed CC smoking rather than exposure to nicotine *per se*. Two studies were identified that had evaluated acute effects of nicotine in young adults. One study evaluated effects of either acute CC smoking or ingestion of a 2 mg nicotine lozenge on exercise heat tolerance test (HTT) and heart rate variability (HRV) in healthy young male volunteers (8 CC smokers and 8 non-CC-smokers, average age around 24 years). In CC smokers, both CC smoking and nicotine lozenge ingestion led to altered HRV, and increased sweat-rate and final core temperature during HTT. No effects of nicotine lozenge ingestion were observed in nonsmokers[1] (Druyan et al. 2017). Another study found that administration of a 4 mg nicotine gum to young adult non-nicotine-users (average age 21 years, no nicotine exposure for at least 1 year) altered auditory processing responses assessed by electrodes on the frontal region of the scalp, with indications that nicotine was associated with reduced habituation and increased ability and willingness to engage with music (Veltri, Taroyan and Overton 2017).

18. A total of 11 citations were flagged that related to studies of adverse effects of nicotine exposure in adolescent animals (rats and mice). Of these, 2 studies described effects of nicotine on cognitive or behavioural outcomes (improved motor function, reduced memory function, decreased anxiety) immediately or shortly after the exposure (Adeniyi et al. 2016, Buck et al. 2017). Two studies described effects of exposure to nicotine during adolescence on longer-term cognitive and behavioural outcomes: enhanced negative occasion setting (Meyer, Chodakewitz and Bucci 2016) and increased spontaneous recovery of fear memory (Zeid and Gould 2018). Five studies investigated effects of adolescent nicotine exposure on consumption of

[1] Effects of CC smoking in nonsmokers were not evaluated.

other drugs, either soon after the nicotine exposures (Singh and Lutfy 2017, Silva et al. 2018) (cocaine, ethanol) or later in adulthood (Stairs et al. 2017, Zipori et al. 2017, Miller, Caruso and Kamens 2018) (d-amphetamine, alcohol, nicotine). One study reported that treatment of male and female rats with nicotine (1.0 mg/kg bw/day by i.p. injection) during adolescence (PND25-PND59), with subsequent mating of treated animals at PND90, led to learning impairments in adult female offspring[2] compared with offspring of rats that had not received nicotine treatment (Renaud and Fountain 2016). One study reported that treatment of adolescent male rats for 28 days with nicotine (0.6 mg/kg bw/day by i.p. injection) altered sperm parameters and induced oxidative stress in the prostate and testes (Budin et al. 2017).

E(N)NDS aerosols

Literature reviewed in previous COT discussion papers

19. Some data relating to adverse effects of E(N)NDS in adolescents were reviewed in the previous COT discussion paper, TOX/2018/24, relevant extracts of which are provided in Annex D. These data are recapped in the following paragraphs.

20. Section 5.1.2.4 'Epidemiological studies of asthma and respiratory symptoms in adolescents' (paragraphs 45–54) of TOX/2018/24 reviewed epidemiological studies of potential respiratory toxicity of E(N)NDS in adolescents (Choi and Bernat 2016, Cho and Paik 2016, Wang et al. 2016, Kim, Sim and Choi 2017, McConnell et al. 2017, Schweitzer et al. 2017). These reports described cross-sectional epidemiological studies (in California, Florida, Hawaii, Korea, Hong Kong) that reported significant associations of E(N)NDS use by adolescents with asthma or respiratory symptoms. The studies were based on data obtained from large-scale surveys, using self-reported data on factors such as E(N)NDS use and CC smoking frequency, asthma diagnosis, and the presence of other respiratory symptoms. Studies reported significantly higher prevalence of asthma and/or other respiratory symptoms in E(N)NDS users compared with non-users, with these associations seen in both CC-smoking and non-CC-smoking E(N)NDS users. With relation to this aspect, the 2018 National Academy of Sciences review, 'Public Health Consequences of E-cigarettes', concluded: "Conclusion 11-4. There is moderate evidence for increased cough and wheeze in adolescents who use e-cigarettes and an association with e-cigarette use and an increase in asthma exacerbations." (NAS 2018). The full text of Section 5.1.2.4 (paragraphs 45–54) of TOX/2018/24 is appended at Annex D.

21. Section 5.1.3 'Oral and periodontal health' (paragraphs 61–62) of TOX/2018/24 described one cross-sectional epidemiological study from Korea that had evaluated oral health in relation to E(N)NDS use in adolescents (Cho 2017). As compared with subjects who had never used E(N)NDS, use of nicotine-free

[2] Males were not tested.

E(N)NDS was associated with tongue and/or inside-cheek pain, while use of nicotine-containing E(N)NDS was associated with increased reporting of cracked or broken tooth. The full text of Paragraphs 61–62 of TOX/2018/24 is appended at Annex D.

22. Section 5.2 'Animal studies' (paragraph 75) of TOX/2018/24 reviewed one study that had evaluated effects of exposure to E(N)NDS aerosol on pulmonary function in an animal model designed to simulate effects of exposure during adolescence (Larcombe et al. 2017). In this study, whole-body exposure of female mice between the ages of 4–12 weeks to E(N)NDS aerosols, with or without nicotine, led to impairments in pulmonary function, without pulmonary inflammation, with some different effects noted depending on the carrier used (PG or VG) and the presence or absence of nicotine in the E(N)NDS liquid. Overall, the authors concluded that VG-based E(N)NDS aerosols induced more severe functional pulmonary impairments than PG-based aerosols, and that there was little effect of the presence or absence of nicotine. The full text of paragraph 75 from TOX/2018/24 is appended at Annex D.

Additional literature

23. Searches were carried out to identify literature relating to toxicity and adverse effects of nicotine in adolescents or young adults, published from 01/04/2018 - 11/12/2018, to update from the date-range covered by searches for the COT discussion paper, TOX/2018/24. A total of 256 citations were identified from searches of the Scopus and PubMed databases.

24. The vast majority of the literature identified related to social and economic aspects of E(N)NDS (for example, use, marketing, attitudes, perceptions of harm, discussions on social media). A number of publications related to studies of the development of dependence on E(N)NDS use and of gateway effects of E(N)NDS use to subsequent likelihood to use and/or become dependent on other recreational drugs or drugs of abuse, such as tobacco products, alcohol, and cannabis. Two studies related to the association of tobacco product use and weight/body mass index (BMI), with some suggestion that adolescents, particularly females, are more likely to be E(N)NDS users if they perceive themselves to be overweight or have weight concerns (Cho et al. 2018a, Bennett and Pokhrel 2018). One study found that, in high school students who had never used tobacco products, initiation of E(N)NDS use was more likely in students with symptoms of ADHD or hyperactivity-impulsivity (HI) (odds ratio, OR = 1.22, 95% CI 1.04 to 1.42 for ADHD; OR = 1.26, 95% CI 1.09 to 1.47 for HI) (Goldenson et al. 2018). Another study followed approximately 3300 adolescents in the U.S. in 9th grade (14–15 years old) over a 2-year period, to evaluate the potential association of polytobacco (CC, E(N)NDS, and hookah) use with mental health outcomes, including depression, anxiety, and ADHD symptoms, and with the use of alcohol, marijuana, and other illicit drugs. Three groups were specified: 'polytobacco use' (CC smoking increased, while E(N)NDS and hookah use decreased during the follow-up period), 'chronic polytobacco use'

(use of all 3 types of product increased during the follow-up period), and controls (did not use any tobacco products). After 24 months of follow-up, both the 'polytobacco use' group and the 'chronic polytobacco use' group had higher levels of use of alcohol and illicit drugs and higher reported levels of depression, anxiety, and ADHD than the control group. 'Chronic polytobacco use' was associated with higher levels of ADHD, but not depression or anxiety, than 'polytobacco use' (Cho et al. 2018b).

25. Two publications related to clinical reports. A case report described an 18-year-old female who developed acute hypersensitivity pneumonitis and acute respiratory distress syndrome associated with E(N)NDS use (Sommerfeld et al. 2018). The other publication described a survey of paediatricians in Canada for reports of injuries related to E(N)NDS use. Of 520 completed questionnaires, 220 incidents were reported, of which 135 related to inhalation and 85 to ingestion. Ingestion incidents mostly occurred in very young children. Inhalation cases were mostly males aged 15–19 years, who used E(N)NDS on average 2–3 days per week, and who sought treatment for nausea/vomiting, cough, throat irritation, or acute nicotine toxicity (Richmond et al. 2018).

26. One study evaluated biomarkers of exposure in adolescent (13–18 years) E(N)NDS users. Urinary levels of benzene, ethylene oxide, acrylonitrile, acrolein, and acrylamide were significantly higher in dual CC and E(N)NDS[3] (n=16) users compared with E(N)NDS[4]-only (n=67) users. Acrylonitrile, acrolein, propylene oxide, acrylamide, and crotonaldehyde levels were significantly higher in E(N)NDS-only users compared with controls who did not use E(N)NDS or CC (n=20). In E(N)NDS-only users, acrylonitrile and acrylamide levels were higher in urine of Always/Sometimes compared with Unsure/Never users of nicotine-containing E(N)NDS, while levels of the other VOCs measured did not differ between these two groupings (Rubinstein et al. 2018).

27. No publications were identified describing evaluations of E(N)NDS in adolescent animal models.

Summary

28. Literature is summarised relating to toxicity of nicotine and of E(N)NDS aerosols in adolescents and young adults, including a recap of relevant data presented in previous COT discussion papers and an overview of literature identified from subsequent updated literature searches.

29. Literature relating to nicotine focussed on neurodevelopmental toxicity in adolescence, including the potential for exposure to lead to long-lasting alterations in cognition, attention, memory, mood disorders, and gateway effects to future drug use and/or addictions. These outcomes have been demonstrated in several studies in

[3] E(N)NDS in this group reported as containing nicotine: Always (60%); Sometimes (40%); Unsure (0%); Never (0%)

[4] E(N)NDS in this group reported as containing nicotine: Always (31%); Sometimes (39%); Unsure (15%); Never (15%).

animal models of nicotine exposure in adolescence. No data were available on direct effects of nicotine exposure in human adolescents, but some similar effects to those observed in animal studies have been implied from studies of adolescent CC smokers.

30. Literature relating to E(N)NDS aerosols was more diverse. Apart from one study that found adverse pulmonary effects of exposure to E(N)NDS aerosols in a mouse model of adolescence, all other literature related to humans. Some cross-sectional epidemiological studies showed increased rates of asthma and respiratory symptoms and adverse oral/periodontal effects in adolescent E(N)NDS users. A large body of literature was identified addressing levels of use and social aspects relating to E(N)NDS use by adolescents and young adults. In addition, some studies looked at the potential of E(N)NDS use to lead to gateway effects for the use of drugs and addictive substances, and some studies looked at the use of E(N)NDS in 'polysubstance' combinations. One study that followed a group of around 3300 adolescents over a 2-year period found that subsequent use of alcohol and illicit drugs and reporting of mental health issues were higher in those who were 'polytobacco' users (CC, E(N)NDS, hookah) than those who were non-users, but this study did not investigate the use of any of the individual tobacco products alone.

Questions for the Committee

31. 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 potential health risks associated with exposure of adolescents or young adults to E(N)NDS aerosols?

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
January 2019

Abbreviations

ACh	Acetylcholine
AChR	Acetylcholine receptor
ADHD	Attention deficit hyperactivity disorder
CC	Conventional cigarette
CI	Confidence interval
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
HI	Hyperactivity – impulsivity
HTT	Heat tolerance test
HRV	Heart rate variability
i.p.	Intraperitoneal
OCD	Obsessive compulsive disorder
ODD	Oppositional defiance disorder
OR	Odds ratio
PAH	Polycyclic aromatic hydrocarbon
PG	Propylene glycol
PND	Postnatal day
SD	Standard deviation
VG	Vegetable glycerine (glycerol)
VOC	Volatile organic compound
TSNA	Tobacco-specific nitrosamine

References

Adeniyi, P. A., A. O. Ishola, B. J. Laoye, B. P. Olatunji, O. O. Bankole, P. D. Shallie & O. M. Ogundele (2016) Neural and behavioural changes in male periadolescent mice after prolonged nicotine-MDMA treatment. Metabolic brain disease, 31**,** 93-107.

Bennett, B. L. & P. Pokhrel (2018) Weight Concerns and Use of Cigarettes and E-Cigarettes among Young Adults. International journal of environmental research and public health, 15**,** 10.3390/ijerph15061084.

Buck, J. M., A. S. Morris, S. J. Weber, J. Raber & J. A. Siegel (2017) Effects of adolescent methamphetamine and nicotine exposure on behavioral performance and MAP-2 immunoreactivity in the nucleus accumbens of adolescent mice. Behavioural brain research, 323**,** 78-85.

Budin, S. B., J. H. Kho, J. H. Lee, A. Ramalingam, F. F. Jubaidi, E. S. Latif, S. Zainalabidin, I. S. Taib & J. Mohamed (2017) Low-dose nicotine exposure induced the oxidative damage of reproductive organs and altered the sperm characteristics of adolescent male rats. Malaysian Journal of Medical Sciences, 24**,** 50-57.

Cho, B. Y., D. C. Seo, H. C. Lin, D. K. Lohrmann, A. K. Chomistek, P. S. Hendricks & L. Timsina (2018a) Adolescent Weight and Electronic Vapor Product Use: Comparing BMI-Based With Perceived Weight Status. American Journal of Preventive Medicine, 55**,** 541-550.

Cho, J., N. I. Goldenson, M. D. Stone, R. McConnell, J. L. Barrington-Trimis, C. P. Chou, S. Y. Sussman, N. R. Riggs & A. M. Leventhal (2018b) Characterizing Polytobacco Use Trajectories and Their Associations With Substance Use and Mental Health Across Mid-Adolescence. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco, 20**,** S31-S38.

Cho, J. H. (2017) The association between electronic-cigarette use and self-reported oral symptoms including cracked or broken teeth and tongue and/or inside-cheek pain among adolescents: A cross-sectional study. PLoS One, 12**,** e0180506.

Cho, J. H. & S. Y. Paik (2016) Association between Electronic Cigarette Use and Asthma among High School Students in South Korea. PLoS One, 11**,** e0151022.

Choi, K. & D. Bernat (2016) E-Cigarette Use Amo ng Florida Youth With and Without Asthma. Am J Prev Med, 51**,** 446-53.

Druyan, A., D. Atias, I. Ketko, Y. Cohen-Sivan & Y. Heled (2017) The effects of smoking and nicotine ingestion on exercise heat tolerance. Journal of Basic and Clinical Physiology and Pharmacology, 28**,** 167-170.

England, L. J., K. Aagaard, M. Bloch, K. Conway, K. Cosgrove, R. Grana, T. J. Gould, D. Hatsukami, F. Jensen, D. Kandel, B. Lanphear, F. Leslie, J. R. Pauly, J. Neiderhiser, M. Rubinstein, T. A. Slotkin, E. Spindel, L. Stroud & L. Wakschlag (2017) Developmental toxicity of nicotine: A transdisciplinary synthesis and implications for emerging tobacco products. Neurosci Biobehav Rev, 72**,** 176-189.

Goldenson, N. I., R. Khoddam, M. D. Stone & A. M. Leventhal (2018) Associations of ADHD Symptoms With Smoking and Alternative Tobacco Product Use Initiation During Adolescence. Journal of pediatric psychology, 43**,** 613-624.

Kim, S. Y., S. Sim & H. G. Choi (2017) Active, passive, and electronic cigarette smoking is associated with asthma in adolescents. Sci Rep, 7**,** 17789.

Larcombe, A. N., M. A. Janka, B. J. Mullins, L. J. Berry, A. Bredin & P. J. Franklin (2017) The effects of electronic cigarette aerosol exposure on inflammation and lung function in mice. American Journal of Physiology - Lung Cellular and Molecular Physiology, 313**,** L67-L79.

McConnell, R., J. L. Barrington-Trimis, K. Wang, R. Urman, H. Hong, J. Unger, J. Samet, A. Leventhal & K. Berhane (2017) Electronic cigarette use and respiratory symptoms in adolescents. American Journal of Respiratory and Critical Care Medicine, 195**,** 1043-1049.

Meyer, H. C., M. I. Chodakewitz & D. J. Bucci (2016) Nicotine administration enhances negative occasion setting in adolescent rats. Behavioural brain research, 302**,** 69-72.

Miller, C. N., M. J. Caruso & H. M. Kamens (2018) Early-adolescent male C57BL/6J and DBA/2J mice display reduced sensitivity to acute nicotine administration. Neuroscience letters, 690**,** 151-157.

NAS. 2018. Public Health Consequences of e-cigarettes. Washington, DC: The National Academies Press.

Polosa, R., C. Russell, J. Nitzkin & K. E. Farsalinos (2017) A critique of the US Surgeon General's conclusions regarding e-cigarette use among youth and young adults in the United States of America. Harm Reduct J, 14**,** 61.

Renaud, S. M. & S. B. Fountain (2016) Transgenerational effects of adolescent nicotine exposure in rats: Evidence for cognitive deficits in adult female offspring. Neurotoxicology and teratology, 56**,** 47-54.

Richmond, S. A., I. Pike, J. L. Maguire & A. Macpherson (2018) E-cigarettes: A new hazard for children and adolescents. Paediatrics & child health, 23**,** 255-259.

Rubinstein, M. L., K. Delucchi, N. L. Benowitz & D. E. Ramo (2018) Adolescent Exposure to Toxic Volatile Organic Chemicals From E-Cigarettes. Pediatrics, 141.

Schweitzer, R. J., T. A. Wills, E. Tam, I. Pagano & K. Choi (2017) E-cigarette use and asthma in a multiethnic sample of adolescents. Preventive medicine, 105**,** 226-231.

Silva, C. P., W. J. Horton, M. J. Caruso, A. Sebastian, L. C. Klein, I. Albert & H. M. Kamens (2018) The influence of adolescent nicotine exposure on ethanol intake and brain gene expression. PloS one, 13**,** e0198935.

Singh, P. K. & K. Lutfy (2017) Nicotine pretreatment reduced cocaine-induced CPP and its reinstatement in a sex- and dose-related manner in adult C57BL/6J mice. Pharmacology, biochemistry, and behavior, 159**,** 84-89.

Sommerfeld, C. G., D. J. Weiner, A. Nowalk & A. Larkin (2018) Hypersensitivity Pneumonitis and Acute Respiratory Distress Syndrome From E-Cigarette Use. Pediatrics, 141**,** 10.1542/peds.2016-3927. Epub 2018 May 17.

Stairs, D. J., S. E. Ewin, M. M. Kangiser & M. N. Pfaff (2017) Effects of environmental enrichment on d-Amphetamine self-administration following nicotine exposure. Experimental and clinical psychopharmacology, 25**,** 393-401.

U.S. Department of Health and Human Services. 2016. Health Effects of E-Cigarette Use Among U.S. Youth and Young Adults. In E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Veltri, T., N. Taroyan & P. G. Overton (2017) Nicotine enhances an auditory Event-Related Potential component which is inversely related to habituation. Journal of Psychopharmacology, 31**,** 861-872.

Wang, M. P., S. Y. Ho, L. T. Leung & T. H. Lam (2016) Electronic Cigarette Use and Respiratory Symptoms in Chinese Adolescents in Hong Kong. JAMA Pediatrics, 170**,** 89-91.

Zeid, D. & T. J. Gould (2018) Chronic nicotine exposure in preadolescence enhances later spontaneous recovery of fear memory. Behavioral neuroscience, 132**,** 240-246.

Zipori, D., Y. Sadot-Sogrin, K. Goltseker, O. Even-Chen, N. Rahamim, O. Shaham & S. Barak (2017) Re-exposure to nicotine-Associated context from adolescence enhances alcohol intake in adulthood. Scientific Reports, 7.

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 8: Additional information on toxicity in adolescent and young adult users.

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

Searches were carried out to identify literature published between 01/01/2016 – 11/12/2018 relating to toxicity of nicotine in adolescents and young adults as follows.

Scopus

(TITLE-ABS-KEY (nicotine) AND TITLE-ABS-KEY (adolescen* OR youth OR teenage* OR "young person" OR "young adult") AND NOT TITLE-ABS-KEY (dependen* OR reward OR addict* OR attitude OR tobacco)) AND PUBYEAR > 2015 AND (LIMIT-TO (LANGUAGE , "English")) AND (EXCLUDE (LANGUAGE , "German") OR EXCLUDE (LANGUAGE , "Portuguese")): 218 citations.

PubMed

(((((((((nicotine[Title/Abstract]) AND ("2016/01/01"[PDat] : "2018/12/31"[PDat]))) AND ((((adolescen* OR youth OR youths OR young adult* OR teenager*[MeSH Terms])) OR (adolescen*[Title/Abstract] OR youth[Title/Abstract] OR youths[Title/Abstract] OR young adult*[Title/Abstract] OR teenager*[Title/Abstract] OR "young person"[Title/Abstract])) AND ("2016/01/01"[PDat] : "2018/12/31"[PDat]))) AND ("2016/01/01"[PDat] : "2018/12/31"[PDat]))) AND (english[Language] AND ("2016/01/01"[PDat] : "2018/12/31"[PDat]))) AND ("2016/01/01"[PDat] : "2018/12/31"[PDat]))) NOT (((((((dependen* [Title/Abstract] OR reward [Title/Abstract] OR addict* [Title/Abstract] OR attitude [Title/Abstract] OR tobacco[Title/Abstract])) OR (nicotine dependence OR dependence, nicotine OR reward* OR attitude* OR tobacco dependence OR dependence, tobacco[MeSH Terms])) AND ("2016/01/01"[PDat] : "2018/12/31"[PDat]))) AND (english[Language] AND ("2016/01/01"[PDat] : "2018/12/31"[PDat]))) AND ("2016/01/01"[PDat] : "2018/12/31"[PDat])): 246 citations.

Searches were carried out to identify literature relating to toxicity of nicotine published between 01/04/2018 – 11/12/2018 in adolescents and young adults as follows.

Scopus

(TITLE-ABS-KEY ("e-cig*" OR "electronic cigarette*" OR "electronic nicotine delivery system*") AND TITLE-ABS-KEY (adolescen* OR youth OR teenage* OR "young person" OR “young adult”)) (PUBDATETXT (april 2018 OR may 2018 OR june 2018 OR july 2018 OR august 2018 OR september 2018 OR october 2018 OR november 2018 OR december 2018)) AND (EXCLUDE (LANGUAGE , "German")): 33 citations

PubMed

((((((e-cig* [Title/Abstract] OR "electronic cigarette*" [Title/Abstract] OR "electronic nicotine delivery system*"[Title/Abstract]) AND ("2018/04/01"[PDat] : "2018/12/31"[PDat]))) AND ((((adolescen* OR youth OR youths OR young adult* OR teenager*[MeSH Terms])) OR (adolescen*[Title/Abstract] OR youth[Title/Abstract] OR youths[Title/Abstract] OR young adult*[Title/Abstract] OR teenager*[Title/Abstract] OR "young person"[Title/Abstract])) AND ("2018/04/01"[PDat] : "2018/12/31"[PDat]))) AND ("2018/04/01"[PDat] : "2018/12/31"[PDat]))) AND (english[Language] AND ("2018/04/01"[PDat] : "2018/12/31"[PDat])): 245 citations.

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 8: Additional information on toxicity in adolescent and young adult users.

Pages 104-107 ‘Effects of Nicotine in Youth Users’ and References list from Chapter 3 of the U.S. Surgeon General Report ‘E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General’ U.S. Department of Health and Human Services 2016).

The full report is available at:

https://e-cigarettes.surgeongeneral.gov/documents/2016_sgr_full_report_non-508.pdf (accessed 12/12/2018).

For copyright reasons the content of this Annex is not included in the published version on the COT website.

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 8: Additional information on toxicity in adolescent and young adult users.

England et al (2017). Developmental toxicity of nicotine: A transdiciplinary synthesis and implications for emerging tobacco products. Neurosci Biobehav Rev, 72, 176-189.

For copyright reasons the content of this Annex is not included in the published version on the COT website.

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 8: Additional information on toxicity in adolescent and young adult users.

Extracts from previous COT paper TOX/2018/24

Reproduction of paragraphs 45-54, 61-62, and 75 from the COT discussion paper, TOX/2018/24 ‘Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 4: Toxicological and epidemiological evaluations of E(N)NDS aerosol exposures.’

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 4: Toxicological and epidemiological evaluations of E(N)NDS aerosol exposures.

5.1.2.4 Epidemiological studies of asthma and respiratory symptoms in adolescents

45. Some cross-sectional studies, based on self-reported information from questionnaire surveys, have observed associations between E(N)NDS use and asthma and/or other respiratory symptoms in adolescents.

46. Analysis of data from the 2012 Florida Youth Tobacco Survey indicated that asthma was more common in adolescents in Florida who were current or previous E(N)NDS users compared with never users (Choi and Bernat 2016). Questionnaire data from high school students (n=36,085) included information on ever diagnosis of asthma and current asthma status, asthma attack during the previous 12 months, and E(N)NDS and/or CC use, ever or within the last 30 days (current). The proportion of overall study population who were ever E(N)NDS users (n=3185) was 8.2% and that of current E(N)NDS users (n=1320) was 3.3%. E(N)NDS use was higher in participants reporting asthma (10.4% had ever used E(N)NDS and 5.3% were current E(N)NDS users) compared with those 'never diagnosed with asthma' (7.2% had ever used E(N)NDS and 2.5% were current E(N)NDS users)[5]. Authors also reported that 'Past 30-day e-cigarette use was associated with having an asthma attack in the past 12 months among participants with asthma (n=5865; $p<0.01$).' E(N)NDS use vs. no E(N)NDS use within the last 30 days was associated with reporting an asthma attack within the previous 12 months (adjusted[6] OR=1.78; 95% confidence interval (CI), 1.20-2.64). However, it is difficult to evaluate the various associations reported by the authors as data are not clearly presented in the publication.

47. Cho and Paik (2016) found an association between asthma and E(N)NDS use in adolescents in South Korea. Data were obtained from the Tenth Korean Youth Risk Behavior Web-based Survey (KYRBWS) in 2014. High school students in 10th-12th grade (n=35,904; mean age 16.4 years) completed a questionnaire including whether they had been diagnosed with asthma by a doctor within the last 12 months,

[5] It is unclear exactly what these percentages represent as the data relating to numbers of study particapants to which they refer are not given in the publication.
[6] Adjusted for age, race/ethnicity, gender, metropolitan status, days smoked cigarettes in the past 30 days, positive social norm towards smoking, and exposure to second-hand smoke.

number of days absence from school with asthma during the past 12 months (as a measure of asthma severity), ever use and current use (within 30 days) of E(N)NDS and of CC. Overall, 674 (1.9%) of the study population had been diagnosed with asthma within the last 12 months. Of these, 98 were current E(N)NDS users (3.9% of n=2513 current E(N)NDS users), 46 were previous E(N)NDS users (2.2% of n=2078 previous E(N)NDS users), and 530 had never used E(N)NDS (1.7% of n=31,313 never E(N)NDS users). Regarding CC smoking, 150 and 524 asthmatics were described as CC smokers and non-smokers, respectively[7].

48. The unadjusted OR for asthma in current vs. never E(N)NDS users was 2.36 (95% CI, 1.89-2.94), adjusted for gender the OR was 2.09 (95% CI, 1.67-2.62), and adjustment for CC smoking produced an OR of 1.73 (95% CI, 1.28-2.34). After stratification for smoking (never, former, current), within the never smokers group the unadjusted OR for asthma in current vs. never E(N)NDS users was 3.41 (95% CI, 1.79-6.49) and the adjusted[8] OR was 2.74 (95% CI, 1.30-5.78).

49. Current E(N)NDS use was also associated with increased school absence due to asthma. Using multi-nominal logistic regression analysis of the frequency of students' absence from school for asthma[9], within the never CC smokers group, ORs for > 4 days absence from school (taken as an indicator of severe asthma) were 18.59 (95% CI, 7.23-47.82) unadjusted and 15.42 (95% CI, 5.11-46.57) adjusted for current E(N)NDS users. For former E(N)NDS users, the ORs were 2.03 (95% CI, 0.28-14.81) unadjusted and 1.63 (95% CI, 0.22-12.15) adjusted.

50. An evaluation of previous KYRBWS data (2011, 2012, 2013) by Kim et al (2017) also indicated a positive relation of E(N)NDS use with asthma (adjusted[10] OR=1.12, 95% CI, 1.01-1.27), although the authors noted that in this analysis, effects of past smoking history could not be excluded.

51. Wang et al (2016) also found that respiratory symptoms were more prevalent in association with E(N)NDS use in Chinese adolescents in Hong Kong. Based on completed questionnaires from the Global Youth Tobacco Survey, data were available from 45,128 students (mean age, 14.6 years), of whom 1.1% had used E(N)NDS within the last 30 days. E(N)NDS use was associated with a higher prevalence of respiratory symptoms (cough or phlegm for 3 consecutive months) overall (adjusted[11] OR=1.28, 95% CI 1.06-1.56), and by breakdown in non-smoker (adjusted odds ratio (AOR)=2.06, 95% CI 1.24-3.42), ever smoker (AOR=1.39, 95% CI 1.14-1.70), and ex-smoker (AOR=1.40, 95% CI 1.02-1.91) groups. The

[7] It is not clear whether 'former smokers' are included in the 'smokers' or 'non-smokers' group.
[8] Adjusted for gender, city size, multi-cultural family status, overweight, second-hand smoking, atopic dermatitis history, allergic rhinitis history.
[9] The reference category was 'no asthma symptom' group.
[10] Adjusted for age, physical exercise, sex, obesity, region of residence, economic level, educational level of father, educational level of mother, active and passive CC smoking.
[11] Adjusted for sex, age, perceived family affluence, second-hand smoke exposure, and school clustering effects.

association was not statistically significant in current smokers (AOR=1.15, 95% CI 0.81-1.62).

52. McConnell et al (2017) reported that E(N)NDS use was associated with increased rates of chronic bronchitic symptoms in Californian adolescents. In 2014, participants in the Southern California Children's Health Study (n=2086) in 11th and 12th grade (mean age, 17.3 years) completed a questionnaire on E(N)NDS use, tobacco product use, and symptoms of chronic bronchitis (chronic cough, phlegm, bronchitis) and wheeze during the previous 12 months. A total of 502 participants had ever used E(N)NDS, of whom 301 were previous users and 201 were current users (24%, 14.4%, and 9.6%, respectively). Compared with never use, bronchitic symptoms were correlated with past (OR=1.85, 95% CI 1.37-2.49) and current (OR=2.02, 95% CI 1.42-2.88) E(N)NDS use, with risk higher with higher frequency of current use (OR=1.66, 95% CI 1.02-2.68 for 1-2 days and OR=2.52, 95% CI 1.56-4.08 for ≥ 3 days use in the previous 30 days). Analysis restricted to never CC smokers indicated an association of bronchitic symptoms with past E(N)NDS use (OR=1.70, 95% CI 1.11-2.59), while results were not statistically significant in current E(N)NDS users/never CC smokers (OR=1.52, 95% CI 0.89-2.61). No association was observed between E(N)NDS use and wheeze.

53. Schweitzer et al (2017) found that E(N)NDS use was associated with asthma in a cross-sectional study of adolescents in Hawaii. Data on E(N)NDS, CC, and marijuana use were collected from the 2015 Hawaii Youth Risk Behavior Survey (HYRBS). A total of 6089 adolescents (50% female, mean age 15.8 y) were included, of whom 22% reported current asthma[12]. For E(N)NDS, 45% were ever users, and 25% of these had used within the last 30 days (current). CC and marijuana were ever used by 25% and 33%, respectively, of whom 10% and 19%, respectively, had used within the last 30 days. In the multivariate analysis, with co-variables including smoking, marijuana use, and other demographic factors, the adjusted OR for the contrast 'current asthma vs. never asthma' in current E(N)NDS users (with 'persons who do not report current use' as the referent group) was 1.48 (95% CI, 1.24-1.78). The adjusted OR for the contrast 'current asthma vs. never asthma' in subjects who had ever used E(N)NDS (never E(N)NDS users were the referent group) was 1.22 (95% CI, 1.01-1.47). Current CC use was not associated with a significantly altered adjusted OR for the contrast 'current asthma vs. never asthma' (referent group, 'persons who do not report current use') (OR=1.23, 95% CI 0.92-1.64), while ever CC use was associated with a significantly increased adjusted OR for 'current asthma vs. never asthma' (never CC use was the referent group) (OR=1.27; 95% CI 1.05-1.54). There were no statistically significant associations between marijuana use and asthma.

[12] Authors noted that these prevalences are high (national average in USA for 2015 being 7.8%), but are close to rates reported for Hong Kong and Florida in the studies of Choi and Bernat (2016) and Wang et al. (2016), respectively.

54. NAS (2018) concluded that 'There is moderate evidence for increased cough and wheeze in adolescents who use e-cigarettes and an association with e-cigarette use and an increase in asthma exacerbations.'

5.1.3 Oral and periodontal health

61. A cross-sectional epidemiological study in Korea evaluated oral health in relation to E(N)NDS use in adolescents (Cho 2017). Data were taken from the 12th Korean Youth Risk Behavior Web-based Survey (KYRBWS), including 65,528 students with a mean age of 15 y. Students were asked to report whether, within the previous 12 months, they had experienced: gingival pain and/or bleeding (18.5%); tongue and/or inside cheek pain (11.0%); a cracked or broken tooth (11.4%). They were questioned on E(N)NDS use and categorised as never users, former users (5.9%) , 1-29 days past month users (1.9%), or daily users (0.5%). Adjusted ORs indicated association of E(N)NDS use with increased incidence of cracked or broken tooth in former (OR=1.16, 95% CI 1.04-1.30, $p<0.05$), 1-29 days past month (OR=1.26, 95% CI 1.06-1.51, $p<0.05$), and daily (OR=1.65, 95% CI 1.65-2.27, $p<0.01$) E(N)NDS users, and with tongue and/or cheek pain in daily users (OR=1.54, 95% CI 1.05-2.26, $p<0.05$) compared with never users. There were no significant differences for reported gingival pain and/or bleeding.

62. Further analysis indicated different effects of nicotine-containing and non-nicotine-containing E(N)NDS. As compared with subjects who had never used E(N)NDS, use of nicotine-free E(N)NDS during the past 30 days was associated with more tongue and/or inside-cheek pain (adjusted OR=1.56, 95% CI 1.07-2.28, $p<0.05$), while use of nicotine-containing E(N)NDS was associated with increased reporting of cracked or broken tooth (adjusted OR=1.16, 95% CI 1.04-1.30, $p<0.05$ for former users; adjusted OR=1.37, 95% CI 1.15-1.63, $p<0.01$ for use within the past 30 days). The authors considered that these findings would be consistent with findings of DNA strand breaks and cell death caused by E(N)NDS aerosol components in in vitro studies (tongue and cheek pain) and known effects of nicotine on tooth structure (cracked or broken tooth).

5.2 Animal studies

75. Exposure to E(N)NDS aerosols led to impairments in pulmonary function, without pulmonary inflammation, in a mouse model designed to simulate effects of exposure during adolescence (Larcombe et al 2017). Groups of 12 female BALB/c mice were whole-body exposed between the ages of 4-12 weeks to control air (AIR), CC smoke (SMOKE), or 1 of 4 E(N)NDS aerosols of 'American Tobacco' flavour, as follows: PG (0-PG), PG + 12 mg/mL nicotine (12-PG), VG (0-VG), or VG + 12 mg/mL nicotine (12-VG). Average measured chamber concentrations were reported as 0.014 g/cm-3 for PG and 0.018 g/cm-3 for VG . Mice were exposed for 1 h/day, 5 days/week from weeks 4-10, then twice daily for 1 h, 5 days/week, during weeks 11 and 12. After 8 weeks of exposure, all treatment groups weighed significantly less than AIR controls, with the lowest weight gains in nicotine-exposed mice . Lung

mechanics and function were assessed 24 h after the final exposure. Mice exposed to E(N)NDS aerosols showed various differences in pulmonary function compared with AIR controls, including decreased airway resistance at functional residual capacity (FRC) (0-PG and 0-VG), increased tissue damping at FRC (all 4 E(N)NDS groups), increased tissue elastance at FRC (0-PG and 0-VG), decreased lung volume and changes in volume dependence of tissue damping and elasticity (0-PG, 0-VG, 12-PG). Methacholine challenge tests showed that SMOKE- and VG-exposed mice were significantly more responsive than AIR- or PG-exposed mice, whether or not nicotine was present in the aerosol. SMOKE but not E(N)NDS exposure was associated with increased pulmonary inflammation (increased BAL cells). Overall, the authors concluded that 1] VG-based E(N)NDS aerosols induced more severe functional pulmonary impairments than PG-based aerosols, and 2] there was little effect of the presence or absence of nicotine.