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). Follow-up to Literature update to mid-2019 – further details of publications in TOX/2019/50.

Introduction

1. At the September 2019 COT meeting, the Committee discussed papers summarising data on aspects relating to the ongoing review of the potential toxicological risks associated from electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (collectively abbreviated to E(N)NDS). Following from discussion of TOX/2019/50 which, based on publication abstracts, provided an update of literature relating to potential toxicological risks from E(N)NDS, Members requested that summaries of some of these publications be provided, based on the full text of the documents. Summaries are provided in the following sections for the 10 requested publications.

Constituents and user exposure

3. Kamilari et al. (2018) reported using Total Reflection X-Ray Fluorescence spectrometry (TXRF) for the quantitative analysis of 6 heavy metals (cadmium, lead, nickel, copper, arsenic, chromium) in 22 commercially available E(N)NDS refill liquids and also in individual constituents used for the production of commercial E(N)NDS liquids (propylene glycol (PG), glycerol, nicotine, and 8 different flavouring agents). Arsenic was not detected in any of the samples, while cadmium was not detected in any of the commercial E(N)NDS liquids. Levels of lead, nickel, copper, and chromium in E(N)NDS liquids were described by the authors as being 'well below the concentrations defined by regulatory authorities for inhalant medicines'. Higher levels of some of the heavy metals were determined in some of the individual components, for example, levels of cadmium were noted to be high in samples of nicotine and of two 'American blend tobacco' flavourings. However, the authors considered that the levels that would be calculated to occur after dilution of these constituents into E(N)NDS liquids should not be of concern.

4. Shin et al. (2018) investigated the potential emission of glass particles to E(N)NDS aerosol from 3 different E(N)NDS devices. Samples at puff number 0-10, 101-110, and 201-210 from a cartomizer containing PG:glycerol liquid were collected on polycarbonate filter and analysed by SEM/EDS. The presence of glass fragments was noted in some of the samples above puff number 100, with emission levels increasing with puff number (highest levels identified in puffs 201-210). Glass

particles were also identified in aerosol samples collected without heating of the coil, which the authors considered to indicate that glass particles could be generated from the cartomizer[1] as well as the coil. Authors concluded that glass particles were generated by the combined effects of the coil and cartomizer, with emissions increasing with increasing use of the E(N)NDS device. The detected glass particles were described as 'threadlike', ranging in thickness from 200 nm to 400 nm, and in length from 500 nm to 4 mm. Authors noted that 'more glass particles were generated from older than newer cartomizers'.

5. Angerer et al. (2019) noted the detection of synthetic cannabinoid (SC) compounds in 10 of 21 E(N)NDS liquids tested, and reported studies of the metabolism and potency of these SC compounds in humans. In the course of EU projects for systematic monitoring of the online market for 'legal high' products ('SPICE', 'SPICE II', 'SPICE II Plus', 'SPICE Profiling'), the authors purchased 21 E(N)NDS liquids from online retailers selling herbal blends between May 2014 and June 2015. Analysis by gas chromatography-mass spectrometry (GC-MS) revealed that 10 of the liquids contained 1 or 2 synthetic cannabinoids: 5F-Cumyl-PINACA (3 liquids); 5F-APINACA (4 liquids); AB-CHMINACA (2 liquids); AB-FUBINACA and AB-PINACA (1 liquid). The study went on to investigate the metabolism of 5F-PINACA and the relative potencies of several different SC compounds. The authors concluding that the presence of the cumyl moiety leads to a relatively potent cannabinoid receptor agonist.

6. Lee, Allen, and Christiani (2019) investigated the presence of endotoxins and glucan in E(N)NDS products, with a focus on examining differences according to the type and flavour of products. E(N)NDS cartridges (n=37) and liquid products (n=38) with the highest nicotine content from the 10 top-selling brands in the US were selected and classified into 4 flavour groups: tobacco, menthol, fruit, and other. Endotoxin and glucan concentrations were above the limit of detection (LOD)[2] in 17 (23%), and 61 (81%) of 75 products tested, respectively. After adjusting for brand and flavour, the mean glucan concentration was 3.2 times higher in cartridges compared with E(N)NDS liquid samples. After adjusting for brand and type of product, glucan concentrations in tobacco- and menthol-flavoured E(N)NDS were 10.4 and 3.5 times higher than concentrations found in fruit-flavoured products.

Human health effects

Users

7. Wills et al. (2019) examined data from the 2016 Hawaii Behavioral Risk Factor Surveillance Survey (BRFSS), including 8087 participants with a mean age of 55 years, of whom approximately 50% were female. In this random-dial telephone survey, participants were asked questions about E(N)NDS use, conventional

[1] The cartomizer consisted of a glass tube, metal ring, and atomizer.
[2] LODs ranged from 0.1–1.6 endotoxin units (EU)/mL for endotoxin and 0.0125–0.2 ng/mL for glucan.

cigarette (CC) smoking, and being diagnosed by a health professional with respiratory disorder (asthma, chronic obstructive pulmonary disease (COPD), emphysema, chronic bronchitis). Rates of E(N)NDS use were 22% (ever-use), 2% (current, some days), and 2% (current, every day). Rates of CC smoking were 38% (ever, >100 CC), 4% (current, some days), and 9% (current, every day). Overall prevalence of asthma and COPD were 17% and 4%, respectively. Multivariable analyses tested associations of E(N)NDS use with the respiratory variables, controlling for smoking and for demographic, physical, and psychosocial variables. Asthma and 'chronic pulmonary disorder' were tested separately. In the whole sample, CC smoking was associated with asthma (adjusted odds ratio (AOR)=1.27, confidence interval[3] (CI) 1.10-1.47). E(N)NDS use was significantly associated with chronic pulmonary disorder (AOR=2.58, CI 1.36-4.89, P<0.01). A statistically significant association was not observed between E(N)NDS use and asthma (AOR=1.27, CI 0.96-1.67). Analysis by CC-smoking status indicated that E(N)NDS use by non-CC-smokers was associated with asthma (AOR=1.33, CI 1.00–1.77, p<0.05), but this association was not seen in E(N)NDS users who were CC smokers (AOR=0.92, 95% CI 0.73-1.15).

8. Alzahrani et al. (2018) examined the potential association between E(N)NDS use, CC smoking, and myocardial infarction (MI)[4] using data from participants in the US National Health Interview Surveys of 2014 (n=36,697) and 2016 (n=33,028). Combining data from both surveys, 2259 participants were current E(N)NDS users, of whom approximately 35% were daily users, while 11,718 participants were current CC smokers, of whom approximately 77% smoked CC daily. Statistical analyses showed the following data for association of product use and MI, compared with never use of the product. E(N)NDS use: AOR[5]=1.06 (95% CI 0.86-1.30) for former use, AOR=1.16 (95% CI 0.83-1.62) for current/some days use, AOR=1.79 (95% CI 1.20-2.66) for current/daily use. CC smoking: AOR=1.70 (95% CI 1.51-1.91) for former use, AOR=2.36 (95% CI 1.80-3.09) for current/some days use, AOR=2.72 (95% CI 2.29-3.24) for current/daily use. History of having had an MI was significantly positively associated with hypertension, diabetes mellitus, high cholesterol, and increasing age, and was negatively associated with female gender, and Hispanic and Asian race. Authors concluded that daily E(N)NDS use, adjusted for smoking CC as well as other risk factors, is associated with increased risk of MI.

9. Osei et al. (2019) found a significantly increased risk of cardiovascular disease (CVD) in dual users of E(N)NDS and CC compared with individuals who were CC smokers only. Authors used pooled data for 2016 and 2017 from the US Behavioral Risk Factor Surveillance Survey (BRFSS) (n=449,092), including current CC smokers (n=58,789), current E(N)NDS users (n=15,863), dual E(N)NDS users and CC smokers (n=12,908). Approximately 10% of participants (n=44,852) reported

[3] The report does not state to which confidence level the term 'confidence interval' refers.
[4] Self-reported, based on the question "Have you EVER been told by a doctor or other health professional that you had a heart attack (also called a myocardial infarction)?".
[5] Adjusted for CC smoking and other risk factors.

CVD[6]. Compared with never users, current E(N)NDS users were more likely to be male, white, heavy alcohol consumers, and CC smokers, but were less likely to have diabetes. Compared with never-CC smokers/never-E(N)NDS users, current E(N)NDS use in never-CC smokers was not significantly associated with CVD (OR=1.04, 95% CI 0.63-1.72). However, compared with CC-smokers/never-E(N)NDS users, current E(N)NDS use in current CC smokers (i.e. dual use) was associated with increased risk of CVD (OR=1.36, 95% CI 1.18-1.56). Further breakdown indicated that the increased risk was present in both occasional and daily E(N)NDS users who were current CC smokers. Analysis for premature CVD (occurring at age < 65 years in females, <55 years in males) indicated a statistically significant increase in current CC smokers who were also current E(N)NDS users (dual users) compared with current CC smokers who were not E(N)NDS users (OR=1.45, 95% CI 1.20-1.74), and breakdown by occasional or daily E(N)NDS use showed significant findings for both groups. Authors considered that these data support the need to conduct longitudinal studies to explore CVD risk associated with E(N)NDS use, particularly among dual users of E(N)NDS and CC.

Bystanders

10. A cross-sectional epidemiological study analysed data from the 2016 Florida Youth Tobacco Survey for potential association between second-hand exposure to E(N)NDS aerosol[7] and having an asthma attack during the previous 12 months[8] in young people with asthma (Bayly et al. 2019). Of the total study population (n = 11,830 young people with a self-reported diagnosis of asthma[9], aged 11–17 years), approximately one-half were female and two-thirds were aged 11–13 years. Between 4–6% were current users of conventional cigarettes (CC), cigars or hookah, while 12% were current E(N)NDS users. One-half were exposed to second-hand CC smoke and one-third to second-hand E(N)NDS aerosol. Asthma attack within the previous 12 months was reported by 21% and showed a statistically significant association with exposure to second-hand E(N)NDS aerosol: AOR[10] =1.27 (95% CI 1.11–1.47) compared with no exposure to second-hand E(N)NDS aerosol. Asthma attack during the past 12 months was also significantly more prevalent in females compared with males (AOR=1.68, 95% CI 1.48-1.90), the 'non-Hispanic other' demographic compared with 'non-Hispanic white' (AOR=2.53, 95% CI 1.20-5.30), current CC users compared with never users (AOR=1.92, 95% CI 1.28-2.68), and second-hand CC smoke exposure compared with no second-hand CC smoke exposure (AOR=1.19, 95% CI 1.05-1.35). Asthma attacks were significantly less

[6] Responded yes to the question "Has a doctor, nurse, or other health professional ever told you had a stroke, myocardial infarction or coronary heart disease?".
[7] Answered 'yes' to the questions "During the past 30 days, were you in the same room with someone who was using electronic vapor products?" and/or "During the past 30 days, did you ride in a car with someone smoking electronic vapor products?".
[8] Answered 'yes' to the question "During the past 12 months, did you have an asthma attack?".
[9] Answered 'yes' to the question "Has a doctor or nurse ever told you that you have asthma?".
[10] Adjusted for demographic characteristics, individual tobacco product use (CC, cigars, hookah, E(N)NDS) and second-hand CC smoke exposure.

prevalent in age group 14–17 years compared with 11–13 years (AOR=0.77, 95% CI 0.68-0.87), and the 'Hispanic' (AOR=0.79, 95% CI 0.68 – 0.93) and 'non-Hispanic black' (AOR=0.80, 95% CI 0.67-0.95) demographics compared with 'non-Hispanic white'. E(N)NDS use by asthmatics did not show a statistically significant association with asthma attack during the previous 12 months: AOR=1.01 (95% CI 0.81-1.25) for ever/non-current versus never use; AOR=0.90 (95% CI 0.71-1.15) for current versus never use.

Animal studies

11. Orzabal et al. (2019) reported that chronic exposure to E(N)NDS aerosol during development causes vascular dysfunction and offspring growth deficits. In this study, Sprague-Dawley rat dams were assigned to 3 groups (Control, e-liquid with no nicotine 'Juice', e-liquid with nicotine 'Juice+Nicotine') and then underwent either a prenatal or prenatal+postnatal exposure protocol to E(N)NDS aerosols, with or without nicotine[11], in vaping chambers[12]. Aerosol exposures were carried out on 5 days/week either during gestation (gestational day (GD) 5–21, 3 h/day) (dams) or throughout gestation plus the early postnatal period (GD 5–21 and postnatal day (PND) 4–10, 2 h/day) (dams and pups). Compared with both the 'Control' and 'Juice' groups, the 'Juice+nicotine' group had significantly reduced fetal weight and crown-rump length. With gestational+postnatal exposure, the 'Juice+Nicotine' group had decreased pup weight at PND 4–10 and decreased crown-rump length on PND 10. Maternal uterine and fetal umbilical blood flow was reduced in the 'Juice+Nicotine' group. Authors concluded that chronic exposure to ENDS aerosols containing nicotine during early development can have deleterious health effects on the exposed offspring, including reduced offspring weight and crown-rump length, associated with a marked decrease in blood flow in both the maternal and fetal umbilical circulation. Thus, chronic exposure to ENDS aerosols containing nicotine can lead to potentially harmful developmental effects in early life.

12. Rahali et al. (2018) investigated the effects of intraperitoneal (i.p.) injection of E(N)NDS liquid (with or without nicotine) on semen parameters. Test liquids (tobacco flavour) comprised 50% PG, 40% glycerol, 5-10% distilled water, 1-5% flavours, and either 0% (E-liquid 0) or 1.8% (E-liquid 18) nicotine. The authors reported that "GC-MS analysis of the main components, which are propylene glycol and glycerol, showed that the composition of the two e-liquids different by the presence of nicotine in the 18 mg/ml sample. A peak corresponding to the flavoring agent diacetin was part of the e-liquid without nicotine." Male Wistar rats (n=8/group) were treated by i.p. injection with either saline, e-liquid without nicotine, or e-liquid containing nicotine, for 28 days. At the end of the treatment period, sperm parameters were assessed and epididymal tissues were fixed for histological analysis. Sperm count and viability were significantly reduced in both E-liquid 0 and E-liquid 18 treatment groups in

[11] E(N)NDS liquid: 80:20 PG:glycerol, with or without 10% (100 mg/mL) nicotine (acclimatisation using 5% nicotine from GD 5–8).
[12] Airflow in the chambers was 2.5 L/min, and one 42 mL puff was dispensed per second.

comparison with saline treated controls. Microscopic analysis showed significant increases in morphological abnormalities, with the most abundant abnormalities found at the level of the flagellum. E-liquid compared with saline treatments were associated with decreased plasma testosterone, and with increased myeloperoxidase granulation and leukocyte count, and decreased percent erythrocytes, in seminal fluid. There were some indications of increased levels of oxidative stress in the epididymis of E-liquid–treated rats compared with the saline-treated group.

Questions for the Committee

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

i. Do any of these more detailed summaries of the available new literature provide important new information of relevance to the Committee's evaluation of the potential health effects of E(N)NDS?

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

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Abbreviations

AOR	Adjusted odds ratio
BRFSS	Behavioral Risk Factor Surveillance Survey
CC	Conventional cigarette
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CVD	Cardiovascular disease
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
EU	European Union
GC-MS	Gas chromatography-mass spectrometry
GD	Gestational day
LOD	Limit of detection
PG	Propylene glycol
PND	Post natal day
SC	Synthetic cannabinoid
SEM/EDS	Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy
TXRF	Total Reflection X-Ray Fluorescence spectrometry

References

Alzahrani, T., I. Pena, N. Temesgen, and S. A. Glantz. 2018. 'Association Between Electronic Cigarette Use and Myocardial Infarction', *American Journal of Preventive Medicine,* 55: 455-61.

Angerer, V., F. Franz, B. Moosmann, P. Bisel, and V. Auwärter. 2019. '5F-Cumyl-PINACA in 'e-liquids' for electronic cigarettes: comprehensive characterization of a new type of synthetic cannabinoid in a trendy product including investigations on the in vitro and in vivo phase I metabolism of 5F-Cumyl-PINACA and its non-fluorinated analog Cumyl-PINACA', Forensic Toxicology, 37: 186-96.

Bayly, J. E., D. Bernat, L. Porter, and K. Choi. 2019. 'Secondhand Exposure to Aerosols From Electronic Nicotine Delivery Systems and Asthma Exacerbations Among Youth With Asthma', *Chest*, 155: 88-93.

CDC. 2006. 'Office on Smoking and Health (US). The Health Consequences of Involuntary Exposure to Tobacco Smoke: A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US).'.

Czogala, J., M. L. Goniewicz, B. Fidelus, W. Zielinska-Danch, M. J. Travers, and A. Sobczak. 2014. 'Secondhand exposure to vapors from electronic cigarettes', Nicotine Tob Res, 16: 655-62.

EPA. 2008. 'Reregistration Eligibility Decision for Nicotine. List B. Case No. 2460. March 2008.'.

Kamilari, E., K. Farsalinos, K. Poulas, C. G. Kontoyannis, and M. G. Orkoula. 2018. 'Detection and quantitative determination of heavy metals in electronic cigarette refill liquids using Total Reflection X-ray Fluorescence Spectrometry', Food and Chemical Toxicology, 116: 233-37.

Laugesen, M. 2015. 'Nicotine and toxicant yield ratings of electronic cigarette brands in New Zealand', *N Z Med J*, 128: 77-82.

Lee, M. S., J. G. Allen, and D. C. Christiani. 2019. 'Endotoxin and [Formula: see text] Contamination in Electronic Cigarette Products Sold in the United States', Environmental health perspectives, 127: 47008.

Lindgren, M., L. Molander, C. Verbaan, E. Lunell, and I. Rosen. 1999. 'Electroencephalographic effects of intravenous nicotine--a dose-response study', Psychopharmacology (*Berl)*, 145: 342-50.

Orzabal, M. R., E. R. Lunde-Young, J. I. Ramirez, S. Y. F. Howe, V. D. Naik, J. Lee, C. L. Heaps, D. W. Threadgill, and J. Ramadoss. 2019. 'Chronic exposure to e-cig aerosols during early development causes vascular dysfunction and offspring growth deficits', Translational research : the journal of laboratory and clinical medicine, 207: 70-82.

Osei, A. D., M. Mirbolouk, O. A. Orimoloye, O. Dzaye, S. M. I. Uddin, E. J. Benjamin, M. E. Hall, A. P. DeFilippis, A. Stokes, A. Bhatnagar, K. Nasir, and M. J. Blaha. 2019. 'Association Between E-Cigarette Use and Cardiovascular Disease Among Never and Current Combustible-Cigarette Smokers', The American Journal of Medicine.

Rahali, D., A. Jrad-Lamine, Y. Dallagi, Y. Bdiri, N. Ba, M. El May, S. El Fazaa, and N. El Golli. 2018. 'Semen Parameter Alteration, Histological Changes and Role of Oxidative Stress in Adult Rat Epididymis on Exposure to Electronic Cigarette Refill Liquid', The Chinese journal of physiology, 61: 75-84.

Shin, J. W., S. H. Jo, K. H. Kim, H. N. Song, C. H. Kang, N. Bolan, and J. Hong. 2018. 'Are glass fiber particles released during the use of electronic cigarettes? Development of a semi-quantitative approach to detect glass particle emission due to vaping', Environmental research, 165: 267-73.

UK-DA. 2007. "Nicotine. Volume 1. Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8(1) of 91/414/EEC. Draft: December 2007." In.

Wills, T. A., I. Pagano, R. J. Williams, and E. K. Tam. 2019. 'E-cigarette use and respiratory disorder in an adult sample', Drug and alcohol dependence, 194: 363-70.

Woolf, A., K. Burkhart, T. Caraccio, and T. Litovitz. 1997. 'Childhood poisoning involving transdermal nicotine patches', Pediatrics, 99: E4.

Yuen, S. T., A. R. Gogo, Jr., I. S. Luk, C. H. Cho, J. C. Ho, and T. T. Loh. 1995. 'The effect of nicotine and its interaction with carbon tetrachloride in the rat liver', Pharmacol Toxicol, 77: 225-30.