

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 from September 2019 COT meeting: updated risk assessments for nicotine exposure from ENDS.

Issue

1. The COT has had a number of discussions on nicotine toxicity as part of this ongoing review. The aim of this current paper is to bring the discussions to a point where the Committee can (a) agree the risk assessment of nicotine exposure for ENDS users and (b) decide which is the best data set to use to evaluate the risk from exposure to nicotine from ENDS for bystanders.

Introduction

2. 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 with electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (collectively abbreviated to E(N)NDS). Following from discussions of [TOX/2019/47](#) in which a health-based guidance value (HBGV) for nicotine was proposed, further follow-up was requested to provide separate risk assessments for nicotine exposure in ENDS users and in bystanders. It was also requested to assess whether any literature was available on effects of intravenous (i.v.) nicotine exposure in non-smokers. These aspects are addressed in the present paper.

Clinical studies of acute effects of nicotine exposure in non-smokers

3. Literature searches were carried out to identify publications reporting studies in which acute effects of nicotine exposure on the cardiovascular system or central nervous system (CNS) were investigated in humans. The search strings are presented at Annex A. The publications identified reported studies in which nicotine was administered via i.v. infusion, subcutaneous (s.c.) injection, intra-nasal spray or drops, oral gum or sublingual tablet, or transdermal patch. For the purposes of this report, only the studies that used i.v., s.c., or intra-nasal routes are included. In addition, one clinical study that was reported in a previous COT discussion paper on E(N)NDS is summarised, which investigated effects of inhalation exposure to nicotine in non-smokers (Hansson et al. 1994). Details of the study by Lindgren et al. (1999), in which nicotine was administered i.v. to conventional cigarette (CC) smokers (but not non-smokers), and which was used by the COT to establish an HBGV for nicotine exposure in smokers, are also included for reference. Study characteristics are summarised in Table 1, below, and in the following narrative.

Table 1. Clinical studies reporting acute cardiovascular or CNS effects of nicotine exposure in non-smokers exposed by i.v., s.c., intra-nasal, or inhalation routes.

Details of the study of i.v. nicotine infusion in CC smokers (Lindgren et al. 1999) used by COT to calculate an HBGV for nicotine exposure for ENDS users are listed in row 1 for comparison.

| Study | Test subjects (males and females unless otherwise specified) | Dose level ($\mu\text{g}/\text{kg bw}$), as stated by authors, or [calculated for this report, assuming 70 kg bw] | Route, duration of exposure | Total dose (mg/subject), as stated by authors, or [calculated for this report, assuming 70 kg bw] | Endpoints evaluated |
|--|---|---|-----------------------------|---|---|
| Lindgren et al. (1999) <i>[CC smokers only]</i> | Smokers (mean, 19 CC/day) (n=14). | 0 3.5 7.0 14.0 28.0 | i.v. infusion, 10 min | [0.25] [0.5] [1.0] [2.0] | Heart rate (HR). Electroencephalogram (EEG). |
| Ghatan et al. (1998) | Non-smokers (n=6). Smokers (n=12). | 0.3 /min (non-smokers). 2.0 /min for 30 min then 0.5 /min for 80 min (smokers). | i.v. infusion, 110 min | [2.3 (non-smokers)] [7.0 (smokers)] | Plasma nicotine. Global and regional cerebral blood flow (CBF). Subjective responses. |
| Swan et al. (2007) | Non-smokers. Smokers (19.8% of study population). - n=110 monozygotic and 29 dizygotic twin pairs | 0.5 /min (non-smokers). 1.0 /min (5-15 CC/day). 2.0 /min (≥ 15 CC/day). | i.v. infusion, 30 min | [1.05 (non-smokers)] [2.1 (5-15 CC/day)] [4.2 (≥ 15 CC/day)] | HR. |
| Soria et al. (1996) | Non-smokers (n=5). Smokers (15-40 CC/day) (n=5). - Mostly males. | [0.0] [10.7] [21.4] | i.v. infusion, 10 s | 0.0 0.75 1.5 | HR, systolic blood pressure (SBP), diastolic blood pressure (DBP). Subjective responses. Addiction-related tests. |
| Foulds et al. (1994) | Non-smokers (n=4). | 8.7 (average) | s.c. injection | 0.0 0.6 | Plasma nicotine. HR. EEG. Subjective effects. |

This is a preliminary paper for discussion. It does not represent the views of the Committee and must not be quoted, cited or reproduced.

| Study | Test subjects (males and females unless otherwise specified) | Dose level ($\mu\text{g}/\text{kg bw}$), as stated by authors, or [calculated for this report, assuming 70 kg bw] | Route, duration of exposure | Total dose (mg/subject), as stated by authors, or [calculated for this report, assuming 70 kg bw] | Endpoints evaluated |
|--------------------------|--|---|-----------------------------|---|---|
| Russell et al. (1990) | Non-smokers (n=6, of whom 3 never-smokers). | 13.25 (mean dose reported across all subjects) 12.23 (mean dose reported across the subset of 3 never-smokers) | s.c. injection | 1.0 (except for 2 lighter-weight subjects who were given a total dose of 0.75 mg per subject) | Plasma nicotine. HR. Subjective responses. |
| Postma et al. (2006) | Non-smokers (n=12). Smokers (n=12). Non-smokers with schizophrenia (n=2). Smokers with schizophrenia (n=7). - Males only | 0 12 | s.c. injection | [0.0] [0.84] | HR. Pre-pulse inhibition (PPI) of startle response. |
| Ettinger et al. (2009) | Non-smokers (n=11). Smokers (5-25 CC/day) (n=13). - Males only | 0 12 | s.c. injection | [0.0] [0.84] | Plasma nicotine. HR, mean arterial BP. |
| Le Houezec et al. (1994) | Non-smokers (< 5 CC/lifetime) (n=12) - Males only | [11.4] | s.c. injection | 0.0 0.8 | Plasma nicotine. HR, blood pressure (BP). Subjective responses. Information processing task. |
| Foulds et al. (1997) | Never-smokers (< 20 CC/lifetime) (n=18). Smokers (mean, 21 CC/day) (n=18). | 4.4 (average) 8.8 (average) | s.c. injection | 0.0 0.3 0.6 | HR, finger pulse volume (FPV). Subjective responses. |
| Perkins et al. (1994) | Never-smokers (n=18, ≤ 20 CC/lifetime). Smokers (n=17, ≥ 15 CC/day for ≥ 1 y). | 0 5 10 20 | Measured-dose nasal spray | [0.0] [0.35] [0.7] [1.4] | Plasma nicotine. HR, SBP, DBP. Performance tests. Cognitive tests. Subjective responses. |

This is a preliminary paper for discussion. It does not represent the views of the Committee and must not be quoted, cited or reproduced.

| Study | Test subjects (males and females unless otherwise specified) | Dose level ($\mu\text{g}/\text{kg bw}$), as stated by authors, or [calculated for this report, assuming 70 kg bw] | Route, duration of exposure | Total dose (mg/subject), as stated by authors, or [calculated for this report, assuming 70 kg bw] | Endpoints evaluated |
|------------------------|--|---|-----------------------------|---|--|
| Perkins et al. (2000) | Never smokers (n=37). Smokers (n=55). | 0 10 20 | Measured-dose nasal spray | [0.0] [0.7] [1.4] | HR, SBP, DBP. Subjective responses. - Outcomes were evaluated in correlation with personality-type testing (sensation-seeking scale). |
| Perkins et al. (2001) | Never smokers (n=19). Ex-smokers (n=17). Current non-dependent smokers (mean 3 CC/day) (n=12). Current dependent smokers (mean 21 CC/day) (n=45). | 0 10 20 | Measured-dose nasal spray | [0.0] [0.7] [1.4] | HR, SBP, DBP, finger temperature. Performance tests. Subjective responses. - Outcomes were evaluated for effects of tolerance. |
| Perkins et al. (2008a) | Young adult non-smokers with between 1 and 10 lifetime smoking exposures (n=58). | 0 10 | Measured-dose nasal spray | [0.0] [0.7] | Cardiovascular outcomes. Subjective responses. - Outcomes were evaluated for correlation with early smoking experience (ESE) responses (questionnaire) and for effects of reinforcement. |

This is a preliminary paper for discussion. It does not represent the views of the Committee and must not be quoted, cited or reproduced.

| Study | Test subjects (males and females unless otherwise specified) | Dose level ($\mu\text{g}/\text{kg}$ bw), as stated by authors, or [calculated for this report, assuming 70 kg bw] | Route, duration of exposure | Total dose (mg/subject), as stated by authors, or [calculated for this report, assuming 70 kg bw] | Endpoints evaluated |
|------------------------|---|--|--|---|---|
| Perkins et al. (2008b) | Young adult non-smokers (≤ 10 lifetime smoking exposures). | 0 5 10 | Measured-dose nasal spray | [0.0] [0.35] [0.7] | Cardiovascular responses. Salivary cortisol concentration. Sensory processing and performance tasks, Subjective responses. - Outcomes were evaluated for correlation with factors including genotypes and gender. |
| Myers et al. (2013) | Non-smokers (< 10 CC ever) (n=25). Smokers (mean, 21 CC/day, 15.8 y) (n=30). | [0] [7] [21] | Measured-dose nasal spray | 0.0 0.5 1.5 | HR, BP. Subjective responses. Executive attention and alerting attention. |
| West and Jarvis (1986) | Non-smokers, mostly male (n=1–8). | [0] [2.1] [29] [57] | Nicotine nasal solution (NNS) (liquid droplet in the nose) | 0 ('pepper' solution) 0.15 2, 4 | Performance tests. |
| Hansson et al. (1994) | Non-smokers (n=15 subjects with positive cough response to capsaicin (respiratory tests); subset of n=5 (cardiovascular parameters)). | [0-9.1 nicotine hydrogen tartrate] | Single-breath inhalation | 0-0.64 nicotine hydrogen tartrate | Electrocardiogram (ECG), HR, SBP, DBP. Cough response (C_2 , C_5). Respiratory resistance (RR). |

Intravenous infusion

4. Lindgren et al. (1999)¹ (see [TOX/2019/47](#)) conducted a single-blind, placebo-controlled crossover study with the aim to establish a dose-response relationship between nicotine and quantitative electroencephalogram (EEG) measures in 14 CC smokers (average 19 CC/day). Subjects abstained from nicotine for ≥ 12 h before test sessions, confirmed by plasma nicotine level <4.0 ng/mL. Caffeine was excluded from the diet. Nicotine was administered during separate test sessions, at doses of 0, 3.5, 7.0, 14.0, and 28.0 $\mu\text{g}/\text{kg}$ bw by i.v. infusion over a 10-min period. Heart rate (HR) and EEG (6 segments) were recorded and auditory oddball task analyses (for analysis of event-related potentials) were conducted at baseline (prior to i.v. infusion) and at intervals through to 130 min after the start of i.v. infusion. Venous blood samples were also taken at intervals during this time period. Analyses were based on repeated measures ANOVA (5 nicotine doses X 11 time points X 4 quadrants for quantitative EEG, 5 nicotine doses X 7 time points for data from the oddball task, 5 nicotine doses X 11 time points for plasma nicotine and heart rate).

5. Plasma nicotine concentrations increased in a time- and dose-dependent manner. Nicotine infusions were associated with increased HR in a dose- and time-dependent manner. Authors described the HR acceleration as 'pronounced' after infusion of the 14.0 and 28.0 $\mu\text{g}/\text{kg}$ nicotine doses. For EEG evaluations, linear, dose-related decreases of delta and theta power were recorded, consistent with increased arousal. A significant effect of higher nicotine doses to decrease theta power was noted. The nicotine X time point interaction was significant for theta power, due to pronounced power decreases during infusion of the higher doses of nicotine. Nicotine increased alpha₂ power and alpha peak frequency in a significant, linear dose-response pattern, with a significant nicotine X time point interaction. There were no significant changes in alpha₁, beta, and auditory oddball P300 parameters, except for a significant interaction of nicotine X time point for beta power. Authors concluded that the arousing effect associated with nicotine infusion was marked in delta and theta bands, with a somewhat weaker relationship with alpha₂. As no non-smoking controls were included in the study, it could not be established whether the arousal effect observed was a reversal of abstinence-related sedation or an 'absolute' arousal increase.

6. Ghatan et al. (1998) assessed the effects of nicotine on regional cerebral blood flow (rCBF) in a group of 6 non-smokers and 12 smokers (> 20 CC/day). Test subjects were allocated to one of three groups. Group A (n=8 smokers) abstained from nicotine overnight. Four underwent abstinence then received an i.v. nicotine infusion (Group A.1, n=4) and four received an i.v. nicotine infusion then abstained from smoking. (Group A.2, n=4)². All eight underwent 12 PET scans during abstinence or infusion. Nicotine was administered at

¹ This study was conducted in CC smokers only. The details are included for comparative purposes.

² Plasma nicotine returned to basal levels in between the nicotine infusion and abstinence test periods.

2.0 µg/kg bw/min for 30 min, followed by 0.5 µg/kg bw/min for approximately 80 min, with the dosing regime designed to avoid nausea or discomfort. For each set of 12 PET scans, participants performed a psychometric task (computerised maze test) during 6 scans, and a sham test during the other 6 scans. For non-smokers (Group B), nicotine was infused at 0.3 µg/kg bw/min during 6 PET scans, after which the infusion was changed to saline during the subsequent 6 scans (participant blinded). The Group B non-smokers performed the maze test during all 12 scans. Plasma nicotine levels were included in statistical analyses for Group B. Subjective responses (visual analogue scale (VAS) for mood and possible aversive affects of nicotine) were also collected. Group C comprised a group of 4 smokers who were evaluated for arterial and jugular venous blood oxygenation during nicotine abstinence and then infusion, in order to evaluate global cerebral blood flow and oxygen consumption (dosing as Group A).

7. Mean plasma nicotine concentrations in Groups A and C were around 6 ng/mL on the study day during abstinence study periods, and 25-28 ng/mL during nicotine infusions. Nicotine was not detected at baseline in plasma of group B, while the peak levels reached during infusion were approximately one-third of those observed in Group A. There were no significant differences in maze test score between nicotine-free and nicotine-infusion conditions in Group A or Group B. Global cerebral blood flow and cerebral oxygen uptake, measured in Group C, did not change significantly from before to after nicotine infusion. However, nicotine elicited rCBF changes that were similar in magnitude in smokers and non-smokers, with decreases in the anterior cingulate cortex and the cerebellum, and concomitant increases in the occipital cortex. The authors considered that these regional changes were induced in areas pertaining to the regulation of mood and attention and to the higher order visual cortex.

8. Swan et al. (2007) monitored heart rate (HR) response during i.v. infusion of nicotine in a study of twin-pairs. The study population comprised 110 monozygotic and 29 dizygotic twin-pairs, of whom 19.8% overall were CC smokers and the rest were non-smokers. Prior to tests, participants abstained from alcohol and recreational drugs for one week and fasted and refrained from tobacco use overnight. Deuterium-labelled nicotine was infused i.v. over a period of 30 min, with dosing based on estimated smoking status: 0.5 µg/kg bw/min for estimated 0-5 CC/day, 1.0 µg/kg bw/min for 5-15 CC/day, or 2.0 µg/kg bw/min for ≥ 15 CC/day. Each twin-pair received the same dose, based on the lower baseline plasma cotinine reading of the two. HR was monitored at baseline, during infusion, and over the 30 min post infusion. Over the 60-min time-course, in all treatment groups, HR increased significantly over time during the infusion to a maximum of +13 beats per minute (bpm) over baseline at 30 min, then decreased post infusion to +4.9 bpm over baseline. There was no significant effect of nicotine dose. Change in HR over the 60-min time-course was associated with smoking status, with percent HR change being higher in smokers compared with non-smokers, and with a significant difference remaining after adjustment for plasma nicotine concentration. Post treatment, non-smokers had a significantly more rapid decline in HR compared with smokers.

9. Soria et al. (1996) investigated effects of acute i.v. nicotine administration in CC smokers (15-40 CC/day) in comparison with non-smokers (n=5/group). Participants abstained from smoking and caffeine for 12 h and from alcohol for 24 h prior to each study day, one-week apart. Test doses of 0.0, 0.75, or 1.5 mg nicotine base were infused i.v. in 1 mL saline over a period of 10 s. Nicotine doses were given in increasing order, with the saline-only treatment randomly placed in the sequence. The protocol was double blind, and tests were repeated on three separate days. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR were measured from 60 min prior to dosing through to 30 min afterwards. Subjective response questionnaires were also administered including questions relating to perceived drug strength, good effects, bad effects, liking and disliking (VAS), and negative aspects such as confusion, fatigue, tension and anger (Profile of Mood States, POMS). Three Addiction Research Centre Inventory (ARCI) tests were applied: Morphine Bazedrine Group (MBG) for positive subjective effects; Pentobarbital Chlorpromazine Alcohol Group (PCAG) for fatigue and sedation; and LSD Group for disorientation and weird feelings. Subjects were also requested to respond once per minute to rate the perceived drug effect strength (0–4). Group differences were determined by 3-way analysis of variance (ANOVA), with Dose and Group as the factors and Time as the repeated measure.

10. For cardiovascular parameters, there were no significant main effects of dose and no group X dose interactions, which the authors considered may be due to small sample size. Smokers and non-smokers showed increased HR and BP 1-5 min after injection of nicotine, but not saline. For HR, the dose X time interaction was significant, with both nicotine doses causing significant increases from 1 min to 15 min post injection compared with saline. The group X time interaction was also significant, with a greater difference between HR at peak compared with later times in non-smokers than in smokers. For SBP, there was no significant main effect or interaction involving dose, but there was an increase during the first 3 min after nicotine but not saline injection in both smokers and non-smokers. There was a significant main effect of time, with highest SBP in smokers and non-smokers measured within 3 min of nicotine injection. DBP levels were higher in smokers than non-smokers (significant main effect of group, irrespective of nicotine treatment). There was a significant nicotine dose X time interaction in smokers and in non-smokers, with increases during the first 3 min after injection compared with baseline, and values then returning towards baseline.

11. For the other measurements: VAS showed significant main effects for dose and time, and for dose X time and group X time interactions (due to 'good' effects reported by smokers); significant main effects of group were found for MBG scale (positive feelings; higher scoring by smokers) and LSD scale (disorientation; higher scoring by non-smokers). Beep response to drug effect indicated significantly stronger effects in smokers and non-smokers with nicotine injections compared with saline, and with strongest effects at 1-5 min post injection.

Subcutaneous injection

12. Foulds et al. (1994) investigated effects of s.c. nicotine administration on plasma nicotine concentration, HR, and electroencephalogram (EEG) in four non-smokers (3 never smokers, 1 ex-smoker). On each of two tests days, one week apart, subjects received two injections, at a 40-min interval, of either saline or 0.6 mg nicotine in saline, in a double-blind crossover design with the dose order counter-balanced. The average nicotine dose per injection was reported as 8.7 µg/kg bw. Subjects abstained from alcohol (24 h), caffeine (3 h), and food (2 h) prior to tests. Blood sampling, HR, and EEG measurements were made before and during the 40 min after each injection. Subjective effects were also recorded.

13. Plasma nicotine peaked at 10 min after nicotine injection, increasing from a baseline average of 0.2 ng/mL to 5.3 ng/mL at 10 min after injection 1, and 8.3 ng/mL at 10 min after injection 2, and reaching a trough value of 2.9 ng/mL just before injection 2. The saline injection did not alter plasma nicotine concentration. HR increased after placebo and nicotine injections. After the first and second nicotine injections, the absolute increases were 14 and 13 bpm, respectively, and the increases over placebo were 8.1 and 7.8 bpm. In parallel with the plasma nicotine peak, HR peaked at 10 min post injection. For EEG, nicotine had no effect on theta, alpha, or beta power, but increased the mean dominant alpha frequency in correlation with plasma nicotine concentration. Three subjects noted dizziness and one subject reported nausea after treatment with the first nicotine injection, as compared with placebo. Authors concluded that one 0.6 mg s.c. injection of nicotine was sufficient to produce changes in plasma nicotine, subjective effects, HR, and dominant alpha frequency in non-smokers. The dose was considered to represent approximately one-half of the plasma nicotine boost obtained by smoking one CC. The effect of nicotine to increase dominant alpha frequency on EEG in non-smokers indicated that this was a primary stimulant effect, suggesting that similar findings in studies of CC smokers may also be due to primary effects of nicotine rather than to reversal of withdrawal.

14. Russell et al. (1990) evaluated effects of s.c. nicotine injection on HR and plasma nicotine concentration in six non-smokers (of whom three were never-smokers). The injected dose of nicotine base was either 0.75 or 1.0 mg, depending on body weight (reported as equivalent to an average dose of 13.25 µg/kg bw). HR and plasma nicotine were monitored at baseline and for 60 min post injection. Plasma nicotine peaked at 15 min post injection at an average of 8.5 ng/mL (0.5 ng/mL at baseline; approximately 4 ng/mL at 60 min). HR peaked at 10 min post injection, with an average boost of +11 bpm, and then declined to around baseline by 45-60 min. Five subjects reported subjective effects, including light-headedness and mild dizziness, between around 5 to 20 min post injection. One subject (never-smoker) reported nausea. A hysteresis plot of HR vs. plasma nicotine for the three never-smoker participants showed concentration-effect relationship shifted to the right (HR began to decrease before plasma nicotine peaked), which the authors considered to indicate the development of acute, partial tolerance to nicotine effects on HR.

15. A study by Postma et al. (2006) investigated the effects of nicotine administration to modify neural pre-pulse inhibition in schizophrenic and non-schizophrenic smokers and non-smokers. Pre-pulse inhibition is a phenomenon whereby reflex response to a startling stimulus is dampened by pre-exposure to a weaker pre-stimulus. The effect is time dependent, with maximum effects generally observed using pre-pulse stimuli up to 120 ms prior to the startle, while longer pre-pulse intervals are, conversely, associated with increased startle response. The test is usually carried out using acoustic stimuli, but this study by Postma and colleagues used tactile stimuli (air puffs directed onto the neck). In total, 22 non-schizophrenic (12 non-smokers, 12 smokers) and 9 schizophrenic (2 non-smokers, 7 smokers) subjects underwent the tests³. Participants abstained from alcohol (24 h) and smoking (12 h) prior to the two testing sessions, which were performed double blind, at a 14-day interval. During session 1, participants received an s.c. injection of 12 µg/kg bw nicotine base, and at session 2, a placebo injection (saline). Startle testing commenced 10 min post injection. Responses were detected by electromyographic (EMG) recording of eye-blink response. Tests were performed using pre-pulse times ('stimulus onset asynchrony', SOA) of 30, 60, and 120 ms prior to pulse, and also without a pre-pulse. PPI was calculated as percentage amplitude response in comparison with pulse-only trials.

16. Effects of nicotine on HR during the procedure were assessed via a 2x3x2 ANOVA [drug X occasion (before injection, 9 min after injection, post PPI testing) X smoking status]. A significant interaction between occasion and drug was determined, and *post hoc* analysis indicated that this reflected a significantly increased HR at 9 min post injection. Effect of nicotine on PPI was evaluated by 2x3x2x2 ANOVA [drug X trial type (-30, -60, -120 ms SOA) x group x smoking status]. Analysis showed main effects of trial type and drug, and a three-way interaction of drug X trial type X group. Further analysis showed that nicotine significantly enhanced PPI at all three SOAs in non-schizophrenic participants, but only at 30 ms in schizophrenic participants. Results indicated an enhancing effect of nicotine on PPI in both groups of participants. Effects of nicotine on latency to response peak were analysed 2x4x2x2 [drug X trial type (-30, -60, -120 ms SOA; no pre-pulse) X group X smoking status], showing a significant main effect of trial type, with shorter latencies in pre-pulse+pulse than pulse-only trials. Further testing of a subset of schizophrenic and non-schizophrenic participants (all smokers) in which functional magnetic resonance imaging (fMRI) was performed during the PPI test paradigm provided indication of increased activation of limbic regions and striatum in both groups after nicotine administration, related to increased hippocampal activity. Authors concluded that nicotine enhances tactile PPI in non-schizophrenic and schizophrenic subjects, with preliminary indications that this effect is modulated by increased limbic activity.

17. Ettinger et al. (2009) carried out a randomised, double-blind, crossover-design study to evaluate the effect of nicotine on antisaccades and prosaccades

³ Selected from an initial cohort of 40 non-schizophrenic and 14 schizophrenic subjects, of whom 16 and 5, respectively, were excluded as they did not present startle responses at initial testing.

responses. Participants, all of whom were male, included 11 non-smokers and 13 smokers (5-25 CC/day). Placebo (saline) or nicotine (12 µg/kg bw base) treatments were given by s.c. injection in random order, one week apart. Participants were abstinent from smoking for 2 h prior to tests. Eye movement parameters were tracked by fMRI imaging whilst conducting a set of antisaccade and prosaccade tasks (visual tasks based on either looking away from or following a coloured dot on a computer screen, with a duration of approximately 8+8 min for each antisaccade+prosaccade task-suite). Procedures were conducted both pre- and post-injection. There was no difference between non-smokers and smokers in task performance levels during practice tasks.

18. Plasma nicotine concentrations prior to and during procedures were measured in a subset of participants, reported descriptively. Levels were higher overall in smokers than in non-smokers, with a greater magnitude of increase on nicotine injection, compared with placebo and over time, in non-smokers than in smokers. Overall, mean arterial BP showed a main effect of group (higher in smokers compared with non-smokers), but with no effect of drug, time, or drug X time. HR showed a significant effect of drug X time, with a significant decrease after placebo injection and non-significant increase after nicotine injection. No other significant main or interaction effects were noted for HR.

19. Analysis of nicotine on saccadic variables was carried out by 2x2x2 repeat-measures ANOVA [time (pre-, post-injection) X drug X group]. For antisaccade latency, a drug X time interaction was noted (faster latency pre- to post-nicotine injection) and there was a main effect of group (faster latency in smokers compared with non-smokers). For prosaccade latency there was a drug X group interaction (indicating faster latency in smokers and slower latency in non-smokers after nicotine compared with placebo injection), but without significant difference between nicotine and placebo in either group. Further, detailed studies at the level of brain function were then performed, with differences between non-smokers and smokers, and within-group heterogeneity, noted.

20. Le Houezec et al. (1994) investigated whether a low-dose s.c. nicotine injection would affect information-processing capability in a group of male non-smokers. Twelve participants (<5 CC/lifetime) underwent three test sessions on separate days, including control (no treatment) [session 1], s.c. injection of saline or 0.8 mg nicotine in saline [sessions 2 and 3, double blind and counter-balanced]. Performance effects, plasma nicotine and cotinine, cardiovascular changes, and subjective responses were monitored during session 1, and then before and after injections during sessions 2 and 3. Average peak plasma nicotine concentration on nicotine injection was 2.9 ng/mL. HR increased significantly post- compared with pre- nicotine injection, with effects lasting for the whole test session (1 h). A significant drug X time interaction was noted for both overall drug conditions and for nicotine compared with saline. Information processing tests showed significant changes in responses associated with nicotine compared with saline and nicotine compared with control. No subjective effects were reported other than clear identification of 'drug strength' for nicotine treatment compared with saline. Authors

concluded that low doses of s.c. nicotine directly affect attention or stimulus processing components of information processing.

21. Foulds et al. (1997) reported a randomised, placebo-controlled, crossover study to evaluate physiological and subjective responses to nicotine in non-smokers compared with smokers. Participants were never-smokers (<20 CC equivalents/lifetime) (n=18) and smokers (≥ 15 CC/day for ≥ 2 y) (n=18). They attended four test days in total, spaced at one-week intervals: one pre-testing evaluation (session 1), then three sessions of test injections (sessions 2-4), for which the order of tests was counter-balanced. At each of sessions 2-4, two s.c. injections of either saline, 0.3 mg, or 0.6 mg nicotine were given, at a 40-min interval. Average nicotine doses per injection were reported as 4.4 and 8.8 $\mu\text{g}/\text{kg}$ bw, and were considered to represent the nicotine dose from smoking one-half or one CC, respectively. Participants abstained from nicotine and alcohol (24 h) and caffeine (2 h) prior to sessions 2-4. Tobacco-withdrawal symptoms (adverse nicotine symptoms score, dysphoria score, hunger, arm pain, craving for a cigarette), mood state (alertness, contentedness, calmness), HR, finger-pulse volume (FPV) and skin conductance level (SCL) were recorded. Statistical tests were made for each nicotine dose compared with saline, and analysis of covariance (ANCOVA) was used to compare nicotine effect between non-smokers and smokers.

22. At session 1, no differences were determined between never-smokers and smokers for mood measures, HR, FPV, or SCL.

23. For smokers, at sessions 2-4, compared with session 1, deterioration in mood and decreased HR were noted prior to injection (i.e. after 24 h smoking abstinence). Nicotine injection did not statistically alter mood score, but, at both nicotine doses, was associated with a significant increase in adverse symptoms, arm pain and cigarette craving. Compared with saline, both the 0.3 mg and 0.6 mg nicotine injection led to significantly increased HR (+4.3 and +7.8 bpm, respectively) and reduced FPV.

24. For never-smokers, compared with saline, 0.6 mg but not 0.3 mg nicotine was associated with significantly decreased mood and increased adverse nicotine symptoms, dysphoria and arm pain. Compared with saline, both 0.3 mg and 0.6 mg nicotine led to significantly increased HR (+2.8 and +7.3 bpm, respectively), and reduced FPV. Mean HR peaked at 12.5 min post injection. Nicotine (both doses) was also associated with a significant decrease in FPV, while SCL was unaffected. Two male never-smokers had to discontinue participation briefly after the second 0.6 mg nicotine injection due to excessive dizziness, nausea and, in one case, vomiting.

25. Comparing effects in never-smokers and smokers, there were significantly greater effects of 0.6 mg nicotine on mood score (reduced alertness) and increased adverse nicotine symptoms in never-smokers compared with smokers, while no differences between the two groups for these effects were noted at the 0.3 mg nicotine dose. However, comparison of changes in physiological measures between the two groups was not reported.

Intra-nasal administration

26. Perkins et al. (1994) investigated differences in subjective, behavioural performance, and cardiovascular responses to a range of nicotine doses as a function of CC smoking status and of the amount of immediately preceding nicotine exposure. Never-smokers ($n=18$, ≤ 20 CC/lifetime) and CC smokers ($n=17$; ≥ 15 CC/day for ≥ 1 y) participated in five test sessions, with abstinence from CC smoking, caffeine, and food overnight before each test day. The protocol was double blind. To evaluate chronic tolerance to nicotine in smokers compared with non-smokers, exposure to the test nicotine dose (either 0, 5, 10, or 20 $\mu\text{g}/\text{kg}$ bw) was administered by measured-dose nasal spray once every 30 min for 2 h. The doses were considered to be similar to the range of nicotine absorbed by smokers after smoking CC. Doses were presented on separate days and the order of dosing was counter-balanced. Subjective (POMS, VAS) and cardiovascular (HR, SBP, DBP) tests were performed 3-7 min after dosing, after which behavioural (finger-tapping speed, hand steadiness, hand tremor) and cognitive (memory recognition, Stroop test⁴) tests were carried out during the next 10 min. Plasma nicotine levels were measured in a subset of participants ($n=4/\text{sex}/\text{group}$) at baseline and 5 min and 15 min after dosing. In addition to the four trials, to evaluate the potential development of acute tolerance to nicotine during test doses 1-4, during each test suite a fifth trial dose of 20 $\mu\text{g}/\text{kg}$ bw was given 30 min after dose 4. Data were analysed by 2x3 mixed ANOVA, each with two between-subject factors (smoking status, gender) and one within-subjects factor (nicotine dose). There were no significant effects of gender, thus results were presented collapsing across females and males for each smoking status group.

27. Plasma nicotine showed a dose-dependent, linear increase⁵. Levels were reported as “reliably reduced by 30% in smokers compared with non-smokers” ($p<0.001$). The authors considered this to reflect faster nicotine clearance in non-smokers, thus ANOVA results were supplemented with regression analyses to include plasma nicotine as an independent measure. Mean baseline HR and DBP were significantly lower in smokers (61.5 bpm, 65.3 mm Hg) compared with non-smokers (70.9 bpm, 70.1 mm Hg), which was considered to be consistent with previous reports of acute tobacco abstinence. Significant, dose-dependent effects of nicotine on HR, SBP, and DBP were observed, but there was no significant interaction of dose X smoking status. However, regression analysis with plasma nicotine as an independent variable showed a significant interaction of plasma nicotine X smoking status on HR response (smaller effect in smokers compared with non-smokers).

28. For measures of chronic nicotine tolerance (4 x dose tests), significant main effects of nicotine were observed for VAS (head rush, jittery, relaxed) and POMS (tension, confusion, fatigue). Dose effects were shifted to the right and dampened for

⁴ Speed of keystroke response to symbols or numbers presented on a video monitor.

⁵ Plasma nicotine concentrations were not reported, but appear from Fig.1 of the publication to range from approximately 1-9 ng/mL (non-smokers) and 2-12 ng/mL (smokers) over the 0-20 $\mu\text{g}/\text{kg}$ bw nicotine dose-range (probably within 5-15 min post-dosing, although this is not clear from the report).

smokers, indicating chronic tolerance. Significant dose X smoking status interactions were observed for all aversive measures (but not for VAS relaxed), indicating tolerance in smokers to aversive effects of nicotine. Conversely, smokers showed greater responses in POMS measures (vigour and arousal). Multiple regression analyses with plasma nicotine as an independent variable indicated an interaction between smoking status and plasma nicotine for most measures, indicating chronic tolerance. In behaviour and cognitive tasks, nicotine generally increased the rate of voluntary (finger tapping, Stroop speed) and involuntary (hand steadiness, tremor) motor movement. Nicotine increased finger tapping in a U-shaped curve, shifted to the right in smokers. There were differences in outcomes for hand steadiness and hand tremor, with the former being equally impaired in smokers and non-smokers, while the latter was reduced in smokers compared with non-smokers. Nicotine improved memory performance in a U-shaped curve, which was shifted to the right in smokers. Regression analyses indicated significant interaction of smoking status X plasma nicotine on tremor amplitude and memory accuracy.

29. Acute tolerance (20 µg/kg bw, 5th dose-challenge test) was observed for effects of nicotine on HR, with response significantly smaller after 4x nicotine compared with placebo pre-challenge. Pre-challenge dose X smoking status interaction was not significant. However, a significant effect of acute tolerance was observed in smokers at all doses compared with placebo, whereas this was not the case for non-smokers. There were no significant effects of acute tolerance from nicotine pre-challenge on BP.

30. Perkins et al. (2000) looked at subjective and cardiovascular responses to nicotine in never-smokers (n=37, mean 8.3 tobacco use/lifetime) and smokers (n=55), with the aim to evaluate potential links with 'sensation seeking scale' (SSS) (personality profiling). Participants abstained from smoking overnight before each test session. On each of three test days, the test dose of nicotine (0, 10, or 20 µg/kg bw) was administered three times (once per 30 min over 90 min) via measured-dose nasal spray. The order of the doses was counter-balanced. Cardiovascular parameters (HR, SBP, DBP) were measured during 3-7 min after administration. Subjective responses (POMS, VAS) were then assessed. Flattened dose-response curves were noted for subjective responses in smokers compared with non-smokers. Some SSS sub-scales, for example 'experience seeking and disinhibition' were correlated with subjective responses to nicotine in non-smokers but not in smokers, which the authors considered may be an indication that sensation seeking is associated with greater initial sensitivity to nicotine subjective effects in nicotine-naïve subjects. There were no group differences in cardiovascular measures.

31. A subsequent study looked at association of chronic tolerance to nicotine with tobacco dependence (Perkins et al. 2001). In this study, participants were attributed to one of four groups: never-smokers (n=19), former dependent smokers (mean of 25 CC for 19 y, then 7 y quit, n=17), current non-dependent smokers (mean of 3 CC/day for 14 y, n=12), and current dependent smokers (mean of 21 CC/day for 20 y, n=45). Nicotine was administered by measured-dose nasal spray. As with

previous studies, analyses of group differences used plasma nicotine as a covariate to correct for differences in nicotine exposure related to dispositional tolerance (difference in drug kinetics). Doses of 0, 10, and 20 µg/kg bw nicotine were tested over three sessions using the same protocol as described by Perkins et al. (2000) (paragraph 30, above). Cardiovascular measures (HR, SBP, DBP, finger skin temperature), subjective responses (VAS, POMS), and performance measures (finger-tapping speed, memory recognition, hand steadiness, rapid information processing) were assessed after each nicotine administration.

32. HR, SBP, and DPB increased significantly with nicotine dose, but there were no effects on skin temperature⁶. There were no significant interactions of group by nicotine dose. Significant group X nicotine dose interactions were noted for nine of fifteen subjective effects. Dependent smokers and non-dependent smokers were tolerant to all nine effects, with no differences between the two groups. Ex-smokers were less tolerant (greater responses than current smokers), and never-smokers showed lower tolerance than ex-smokers. For some performance tests, tolerance was seen in dependent smokers but not other groups. Authors considered the findings of equivalent tolerance to subjective effects in dependent and non-dependent smokers to indicate that there is no close link between nicotine tolerance and dependence, thus raising a question as to the utility of tolerance as a criterion for defining dependence.

33. In another study, Perkins et al. (2008a) looked at associations of early smoking experiences (ESE) with subsequent sensitivity of responses to nicotine challenge in a group of 58 young adult non-smokers. Questionnaires were administered to collect data on responses to ESE, then prospective tests were carried out to assess 'nicotine spray effects' (NSE), including cardiovascular responses and subjective effects (VAS). On the first two test days, nicotine was administered by measured-dose nasal spray at 0 or 10 µg/kg bw, with protocols similar to previous studies by Perkins and colleagues, as described in the preceding paragraphs. Two of six subjective measures reported on ESE were correlated with greater responses in NSE tests ('dizzy' and 'buzzed'). Cardiovascular outcomes were not related to ESE responses, other than a significant association of ESE nausea with higher DBP response to nicotine challenge.

34. On the third test day, participants undertook reinforcement tests. They first underwent nicotine self-dosing choice sessions, in which they were pre-exposed to colour-coded nicotine and placebo sprays (but blinded to nicotine content) and then given four sessions where they could choose to self-administer any combination of eight sprays from the two colour choices. Nicotine was chosen less than 50% of the time, and reinforcement was not found to be associated with ESE measures in this group of non-smokers.

35. Perkins et al. (2008b) investigated the influence of genetic variation on responses to nicotine exposure in young adult non-smokers. Participants (n=101,

⁶ Results were not presented for skin temperature.

≤10 lifetime tobacco exposures) were administered nicotine by measured-dose nasal spray at doses of 0, 5, and 10 µg/kg bw⁷ as follows: one dose-level tested per study day, administered 3x (every 30 min over 90 min). Each individual dose was given as eight sprays over a 2-min period (two per 30 s). Mean plasma nicotine after the 5 and 10 µg/kg bw dosing was 2.3 and 3.4 ng/mL, respectively. The order of doses was counter-balanced. After each test dose, subjects were tested for subjective responses ('Positive And Negative Affect Scale' (PANAS), POMS, VAS), cardiovascular responses (HR, SBP, DBP), sensory processing (startle tests), and performance measures (finger-tapping speed, hand steadiness, rapid information processing, memory recall). Salivary cortisol levels were also measured. A choice session was also carried out, similar to that described in Perkins et al. (2008a) (paragraph 34, above). Subjects were genotyped for SNPs at six loci relating to signalling pathways in the CNS. Differences in responses were analysed by analysis of covariance (ANCOVA).

36. There were no significant effects of gene X dose or of gene X dose X sex for cardiovascular responses to nicotine. An interaction of dopamine D4 receptor (DRD4) allele X dose was seen for changes in salivary cortisol concentration. DRD4 was also associated with greater aversive responses to nicotine and reduced nicotine choice. Some between-gender differences were observed in dopamine receptor allele-associated responses (DRD4 and DRD2). Authors commented that these preliminary results suggest that polymorphisms related to function in the dopamine D4 and perhaps D2 receptor may modulate initial sensitivity to nicotine prior to onset of dependence and may do so differentially between men and women.

37. Myers et al. (2013) investigated acute effects of nicotine nasal spray in non-smokers (n=25, < 10 CC/lifetime) and smokers (mean 21 CC/day for 15.8 y) (n=30). Nicotine doses of 0, 0.5, and 1.5 mg were tested in randomised order, on different days. Participants abstained from alcohol and 'other drugs' for 24 h prior to test days, but not from caffeine, nicotine, or prescription drugs. Prior to and post dosing the following evaluations were performed: cardiovascular effects (HR, BP); subjective responses; executive attention tests; alerting attention tests. Nicotine was reported to significantly increase BP in non-smokers and smokers at 5 min post dosing, and to produce a dose-related increase in HR. There was no difference in the magnitude of cardiovascular effects between non-smokers and smokers⁸. Nicotine enhanced alerting attention in non-smokers and in smokers, but did not affect executive attention. Nicotine dosing was significantly associated with several subjective effects (VAS) including stimulation, jittery, and dizzy. *Post hoc* tests indicated effects were observed primarily in non-smokers, although the highest nicotine dose was also associated with dizziness in smokers. Nicotine was significantly associated with 'liking' in non-smokers. Authors concluded that acute administration of intra-nasal nicotine improved alerting attention in non-smokers and

⁷ The 5 and 10 µg/kg bw doses were considered to represent smoking approximately one-quarter and one-half of a CC, respectively.

⁸ Results were not presented.

smokers, and commented that cognitive enhancement might be one reason why people decide to take up smoking.

38. West and Jarvis (1986) studied the acute effects of nicotine applied intra-nasally on finger tapping rate. Five sets of tests were performed in small groups of non-smoking adults (mostly males). Nicotine was administered as a single drop in the nose ('nasal nicotine solution', NNS), associated with peak plasma concentration at 7-10 min post dosing. Tests were mostly conducted in a randomised and double-blind manner.

39. In experiment 1 (n=8 subjects), administration of 4 mg NNS led to a significant 4.2% increase in finger-tapping rate at 10 min after, as compared with 10 min before, dosing. There was no significant effect of placebo treatment (a 'pepper' solution) (0.7% increase). Experiment 2 tested two NNS doses, 2 mg and 0.15 mg, as well as placebo (n=8 subjects). The 2 mg dose was associated with significantly increased (5%) finger-tapping rate compared with pre-dosing. No effects were noted with 0.15 mg NNS or placebo. In experiment 3 (n=5 subjects), two sets of tests were carried out; before and 2 h after administration of mecamylamine (a central cholinergic blocking agent) or placebo (there was no placebo control for NNS treatment in these tests). NNS increased finger-tapping rate in all cases. There was no significant difference in NNS-related increase in finger-tapping rate between pre- and post-treatment with placebo. However, the increase in finger-tapping rate was significantly lower post- compared with pre-mecamylamine treatment. In experiment 4, one test subject performed the finger-tapping test repeatedly at short intervals over 60 min after administration of 2 mg NNS, and, on a different day, after administration of placebo. Finger-tapping rate increased after NNS treatment, remained elevated until 30 min post treatment then declined to a rate that was still above baseline at 1 h. No effects were noted with placebo treatment. Finally, experiment 5 tested potential acute tolerance to effects of NNS to increase finger-tapping rate. Two subjects performed finger-tapping tests before and after administration of 2 mg NNS, with the dosing/testing schedule repeated seven times, once per hour. NNS increased finger-tapping rate in all cases, with no difference in effect seen over time, suggesting no development of acute tolerance to NNS treatment. Authors concluded that NNS provides an effective means of delivering nicotine in sufficient amounts to produce strong and consistent effects on a simple motor task, and that nicotine can substantially improve performance by non-smokers on such a task, probably via its action on cholinergic pathways.

Inhalation

40. Hansson et al. (1994)⁹ investigated acute effects of inhalation exposure to nicotine in non-smokers on cough response, respiratory resistance, and cardiovascular parameters in healthy never-smokers.

41. Single-breath exposures to 0.01 mL nebulised nicotine hydrogen tartrate

⁹ This publication was summarised in a previous COT discussion paper, TOX/2019/2019/38.

solution at concentrations (nicotine salt) in the range of 0-64 mg/mL¹⁰ were tested in 15 subjects with positive cough response to nebulised capsaicin. Thirteen of these 15 subjects showed positive, concentration-dependent cough response to nicotine. Mean (95% confidence interval (CI)) concentrations causing two coughs (C₂) or five coughs (C₅) were 5.5 (3.5-8.7) mg/mL and 15.8 (10.0-25.1) mg/mL, respectively, while C₂ for capsaicin was 7.2 (3.9-13.5) mg/mL. Sensitivities to nicotine and capsaicin were similar when challenges were repeated on two separate days, and also when the order of capsaicin/nicotine challenge tests (conducted 15 min apart) was inverted. Respiratory resistance on challenge with a sub-tussive (< C₂) concentration of test product was increased to a similar extent by single breaths of nicotine and of capsaicin, with the increased resistance lasting for around 2-3 min. Repeats of the tests after 30 min produced results of equivalent magnitude. Five subjects were evaluated for cardiovascular effects of single-breath inhalations of nicotine up to 64 mg/mL, taken at 0 and 10 min. No effects were observed on electrocardiogram (ECG), BP, or HR during the 5 min following each inhalation, but adverse subjective effects were reported (headache, mouth discomfort).

42. In a separate assessment, cough, cardiovascular effects, and skin temperature were evaluated in a double-blind manner in eight participants who inhaled nicotine solutions of 0, 2, 4, or 8 mg/mL on four different days. A single breath was taken every 15 s up to 5 min (total 21 inhalations), giving a total dose of 0, 0.4, 0.8, or 1.7 mg nicotine per 5 min. Measurements were made from 10 min before challenge to 30 min afterwards. HR and SBP increased significantly at all doses during the 30 min after challenge, in a dose-related manner, compared with the vehicle-exposed controls. Maximal responses were seen within 3 min after nicotine inhalation, and the responses lasted between 6 and 10 min. There were no significant changes in DBP. Both nicotine and capsaicin treatments caused a decrease in skin temperature, with maximal response at 5 min. Seven of the subjects complained of headache, which reached a maximum at 5-6 min and lasted for 20 min. None of the subjects noticed a tremor or nausea (Hansson et al. 1994).

Follow-up risk assessment of nicotine exposure for users and bystanders

Summary of health-based guidance values and evaluations for nicotine

43. The COT has previously considered discussion papers reviewing nicotine toxicity ([TOX/2018/25](#), [TOX/2019/38](#)) and the specific areas of potential developmental toxicity via parental exposure ([TOX/2018/45](#)) or from exposure during adolescence and/or early adulthood ([TOX/2019/01](#)). The Committee has previously considered HBGVs for nicotine exposure set by EFSA (EFSA 2009), EU (UK-DAR 2007), and EPA (EPA 2008). The Committee determined that data from the study of Lindgren et al. (1999), as used by EFSA, would be the most appropriate to use in establishing an HBGV for inhalation exposure to nicotine from ENDS.

¹⁰ Tests were carried out firstly by increasing nicotine concentration, and later, in random order.

44. At the September 2019 COT meeting ([TOX/2019/47](#)), the Committee discussed the study of Lindgren et al. (1999) (summarised in paragraphs 4-5), in which HR and EEG parameters were monitored in CC smokers administered nicotine by i.v. infusion (0.0, 3.5, 7.0, 14.0, and 28.0 µg/kg bw, over 10 min), following a 12-h abstinence from smoking. EFSA (2009) had established an acute reference dose (ARfD) and acceptable daily intake (ADI) for oral nicotine exposure from this study based on a lowest adverse effects level (LOAEL) of 3.5 µg/kg bw for effects on HR. The COT concluded that this study could be used to establish an HBGV for nicotine exposure in ENDS users. From the data presented on EEG, the Committee considered that a no observed adverse effect level (NOAEL) in the range of 7–14 µg/kg bw should be used as a point of departure, albeit with some uncertainty. Taking the value of 7 µg/kg bw, applying an adjustment of 0.55 for bioavailability (extrapolation from i.v. to inhalation route)¹¹, and an uncertainty factor (UF) of 10 to account for human variability, this would result in an HBGV of 1.3 µg/kg bw for acute inhalation exposure to nicotine of ENDS users. Given that nicotine has a short biological half-life in humans, does not accumulate in the body, and the most sensitive effect is considered to be pharmacological effects (alterations in EEG) after i.v. infusion, the HBGV established for acute effects of nicotine could also be considered to protect against longer term effects, thus the HBGV for chronic exposure of ENDS users would also be 1.3 µg/kg bw/day.

45. At the September 2019 meeting, it was agreed that as the Lindgren study did not include non-smokers, it was not an appropriate basis for an HBGV to protect bystanders from the effects of nicotine in air following use of ENDS products. The Secretariat proposed to use a margin of exposure (MOE) approach with data from the Lindgren study in the interim, but Members will wish to consider the information provided in paragraphs 6-42 above as to whether there is an alternative appropriate point of departure (POD) for establishing an HBGV for bystanders.

Evaluation of risk to ENDS users

46. Data on potential levels of exposure of users to nicotine from ENDS were summarised in [TOX/2019/39](#) and [TOX/2019/59](#). Study data for all experimental studies which had reported measurements of nicotine and nicotine-related alkaloids were tabulated in Table 4 of [TOX/2019/39](#). The data for nicotine are reproduced in Table 2, below.

¹¹ See [TOX/2019/38](#) for details on bioavailability of nicotine via different routes of exposure

Table 2. Levels of nicotine measured in E(N)NDS aerosols. Data are taken from Table 4 of the COT discussion paper, [TOX/2019/39](#), where further details of the reference cited can be found.

(Nicotine levels in $\mu\text{g}/\text{puff}$, either as reported in the original publications, or calculated from the original data provided, have been highlighted in bold for the purpose of this report.)

| Study | E(N)NDS product(s) and nicotine content Puff volume | Amount of nicotine collected from aerosol and/or concentration in aerosol (unless otherwise stated) [conversion for this paper] |
|--------------------------|--|--|
| Trehy et al. (2011) | 4 products purchased via internet; 16 mg/cartridge nicotine (label), 21 mg/cartridge nicotine (measured); 100 mL | 50–292 $\mu\text{g}/30$ puffs [0.67–9.73 $\mu\text{g}/\text{puff}$, 6.7–97.3 mg/m^3] |
| Pellegrino et al. (2012) | Italian-brand E(N)NDS; 0.25% nicotine | 6.21 mg/m^3 |
| Czogala et al. (2014) | 3 E(N)NDS products purchased in Poland; 18 mg/cartridge nicotine (label), 11–19 mg/cartridge nicotine (measured); 70 mL | 2.51 $\mu\text{g}/\text{m}^3$ (mean) and 0.82–6.23 $\mu\text{g}/\text{m}^3$ (range) in ambient air in a 39 m^3 chamber into which 7 x 1.8 s puffs were emitted |
| Tayyarah and Long (2014) | 2 disposable and 3 rechargeable E(N)NDS; 16–24 mg/unit nicotine (label), 11.7–20.6 mg/unit nicotine (range of mean values for 5 product types) (measured); 55 mL | 8–33 $\mu\text{g}/\text{puff}$ [145–600 mg/m^3] (range of mean values for 5 product types) |
| Laugesen (2015) | 14 E(N)NDS products (9 cigalikes, 3 disposables, 2 cartomizers) from China, UK, and USA; 14.5–23 mg/mL nicotine (label), 11.5–27.4 mg/mL nicotine (measured); 70 mL | 43 $\mu\text{g}/\text{puff}$ [614 mg/m^3] (mean); 18–93 $\mu\text{g}/\text{puff}$ [275–1329 mg/m^3] (range) |
| Flora et al. (2016) | 4 E(N)NDS products of ‘MarkTen’ brand (USA); 1.5% nicotine; 55 mL | 29 $\mu\text{g}/\text{puff}$ [527 mg/m^3] (average) |
| Margham et al. (2016) | Vype ePen (closed modular system with cartomizer, operated at 3.6 V), ‘Blended Tobacco’ E(N)NDS liquid; 1.86% nicotine; 55 mL | 32 $\mu\text{g}/\text{puff}$ [582 mg/m^3] (mean) |

| Study | E(N)NDS product(s) and nicotine content Puff volume | Amount of nicotine collected from aerosol and/or concentration in aerosol (unless otherwise stated) [conversion for this paper] |
|---|--|---|
| Talih et al. (2016) ['direct dripping'] | NHALER 510 Atomizer with ego-T battery (3.4 V); PG-based E(N)NDS liquid, with flavour; E(N)NDS use by "direct dripping" of E(N)NDS liquid (dripping every 2, 3, or 4 puffs); 0 or 18 mg/mL nicotine; 152 mL | 740–1030 µg/15 puffs [49.3–68.7 µg/puff, or 324–451 mg/m ³] (mean); [620–2950 µg/15 puffs [41.3–197 µg/puff, or 272–1294 mg/m ³] (range) |
| Baassiri et al. (2017) | Vapor-Fi second-generation tank system; 18 mg/mL nicotine (PG/glycerol mixtures ranging from 0/100 to 100/0) 67 mL (4 s puffs, 16.7 mL/s flow rate) | 0.13 mg/15 puffs (0/100 PG/glycerol liquid) 0.58 mg/15 puffs (100/0 PG/glycerol liquid) [9–39 µg/puff; 129–577 mg/m ³] |
| Lee et al. (2017) | V2 'cigalike' cartomizer devices (VMR Products): tobacco flavour, menthol flavour; 1.8% nicotine; (2 puffs/min diluted 1:172 into chamber) | Tobacco-flavoured, 4.35 µg/m ³ (mean); Menthol-flavoured, 2.40 µg/m ³ (mean) |

47. The highest levels were reported from the study of Laugesen (2015)¹², which measured a mean nicotine level of 43 µg/puff (range 18-93 µg/puff) in aerosols produced from a range of ENDS products with measured nicotine concentrations in the liquid of 11.5–27.4 mg/mL. Based on these data:

- For a 70 kg user taking 15 puffs during one ENDS-use session¹³, average (range) exposure would be: 9.2 (3.9-19.9) µg/kg bw.
- For a 70 kg user taking 272 puffs/day¹⁴, average (range) daily exposure would be: 167 (70-361) µg/kg bw/day.
- For a 70 kg user taking 338 puffs/day, average (range) daily exposure would be: 208 (87-449) µg/kg bw/day.

¹² Excluding the higher levels reported from the study of Talih et al. (2016), which used a 'direct dripping' method to produce aerosol.

¹³ 15 puffs is suggested as a possible scenario for one ENDS-use session, although it is acknowledged that patterns of use vary between users.

¹⁴ The study of Dawkins et al. (2018) [described in [TOX/2019/39](#)] reported mean daily ENDS use levels with different product-type/nicotine levels as follows: 338 puffs/day (fixed power, 6 mg/mL nicotine), 308 puffs/day (variable power, 6 mg/mL nicotine), 279 puffs/day (fixed power, 18 mg/mL nicotine), and 272 puffs/day (variable power, 18 mg/mL nicotine). The lower and higher values of this range have been used for the user exposure calculations in paragraph 47.

48. The estimated mean levels of exposure to nicotine from one 15-puff ENDS-use session calculated in paragraph 47 would exceed the HBGV of 1.3 µg/kg bw by approximately 7-fold. The estimated mean levels of daily exposure to nicotine from ENDS use calculated in paragraph 47 would exceed the HBGV of 1.3 µg/kg bw/day by approximately 130-fold (272 puffs/day) to 160-fold (338 puffs/day).

49. An average daily exposure to nicotine from smoking CC has been calculated as 500 µg/kg bw/day (see [TOX/2019/39](#) for details). This level of nicotine exposure exceeds the HBGV of 1.3 µg/kg bw/day by approximately 385-fold. The estimated mean levels of daily exposure to nicotine from ENDS use calculated in paragraph 47 represent approximately 33-42% of this average daily exposure from CC smoking.

Evaluation of risk to bystanders

50. Data on levels of nicotine measured in ambient air associated with use of E(N)NDS products were presented in the COT discussion paper, [TOX/2019/11](#), and are summarised in Table 3, below.

Table 3. Studies listed in TOX/2019/11 that reported measurements of nicotine in ambient air where E(N)NDS products were being or had been used.

| Reference | Study details | Average nicotine level measured in ambient air (mean \pm SD (range)), $\mu\text{g}/\text{m}^3$ |
|--|--|--|
| <i>Experimental studies in rooms or exposure chambers</i> | | |
| Saffari et al. (2014) | 48 m ³ room, 1 user, 7 puffs/10 min, 16 mg/mL nicotine, air-exchange 1.1/h | 0.123 |
| Melstrom et al. (2017) | 52.6 m ³ room, 3 users, <i>ad libitum</i> for 2 h, 12-20.5 mg/mL nicotine, air exchange 5/h | 0.717 (0.445-0.989) (disposable) 1.680 (1.158-2.047) (tank) |
| Schober et al. (2014) | 45 m ³ room, 3 users, <i>ad libitum</i> for 2 h, 18 mg/mL nicotine, air exchange 0.37-0.74/h | 2.2 \pm 1.7 |
| Liu et al. (2017) ¹ | 114 m ³ chamber, 8 users, 80 puffs/user over 4 h, 2.4% nicotine, air exchange 7.5 L/s | 2.83 |
| Czogala et al. (2014) ² | 39 m ³ chamber, 1 user, 2 x 5-min <i>ad libitum</i> at 30-min interval (1 h mean level measured), 16-18 mg/mL nicotine, air exchange – not reported | 3.32 \pm 2.49 (0.65-6.23) |
| O'Connell et al. (2015) ³ | 38.5 m ³ room, 3 users, 165 min, 16 mg/g nicotine, air exchange 0.8/h | < 7 (LOD) |
| Maloney et al. (2016) ⁴ | 137 m ³ room, 2-12 users, 6 x 1 hour, 1.5-2.5% nicotine, air exchange 1.47-1.56/h | < 10-15 (LOQ) |
| <i>Other settings</i> | | |
| Ballbe et al. (2014) | Main family rooms | 0.02 (non-E(N)NDS-use households) 0.13 (E(N)NDS-use households) |

This is a preliminary paper for discussion. It does not represent the views of the Committee and must not be quoted, cited or reproduced.

| Reference | Study details | Average nicotine level measured in ambient air (mean \pm SD (range)), $\mu\text{g}/\text{m}^3$ |
|-----------------------|--|--|
| Schober et al. (2019) | Moving car, 1 user (front of car), <i>ad libitum</i> use, 14 test sessions, air sampled in back of car, 18 mg/mL nicotine, windows open 2-5 cm | < LOD (not specified) in 8 test sessions 4-10 in 6 test sessions |
| Johnson et al. (2018) | 4 indoor vaping events, described as 'well ventilated' | 1.1 (0.36-2.2) |
| Chen et al. (2017) | Indoor vaping event, described as 'poorly ventilated' | 124.7 (109.2-140.2) |

¹ The 'Conflicts of Interest' section of the publication states that "The study was funded by Altria Client Services LLC. The authors, Mohamadi Sarkar, Jianmin Liu, Qiwei Liang, Michael J. Oldham, Ali A. Rostami and Karl A. Wagner are employees of ALCS. I. Gene Gillman, Piyush Patel and Rebecca Savioz are paid contractors. The study was conducted on behalf of NuMark LLC., (Richmond, VA, USA) a subsidiary of Altria Group, that produces and markets e-vapor products."

² Authors reported: "MLG received research funding from Pfizer, manufacturer of stop smoking medication, and was funded by the UK Centre for Tobacco Control Studies (UKCTCS) during the study. AS received research funds and travel expenses from Chic Group Ltd., manufacturer of electronic cigarettes in Poland. Other authors declare no conflicts of interest". The study was funded by the Ministry of Science and Higher Education of Poland.

³ The 'Conflicts of Interest' listing of this publication states that "All authors are employees of Imperial Tobacco Group. The work in this manuscript was supported by Imperial Tobacco Group. Imperial Tobacco Group is the parent company of Fontem Ventures B.V., the manufacturer of the e-cigarette products used in this study."

⁴ The 'Funding' acknowledgement section of this publication notes that "All authors of this study are current or retired employees of Altria Client Services which is a subsidiary of Altria Group. NuMark, a subsidiary of Altria Group, is a manufacturer of electronic cigarettes. Funding for this project was provided by Altria Client Services."

51. Considering the data obtained from studies reported in [TOX/2019/11](#) that measured nicotine levels in association with ENDS use under pre-specified conditions in rooms or exposure chambers, the highest mean (range) ambient air nicotine level associated with ENDS use was 3.32 (0.65-6.23) $\mu\text{g}/\text{m}^3$ reported by Czogala et al. (2014). Based on these data:

- For a 70 kg individual inhaling 20 m^3 air during 24 h, this would lead to a nicotine intake of 0.95 (0.19-1.78) $\mu\text{g}/\text{kg}$ bw/day.
- For a 13.3 kg, 1-6 year-old child inhaling 8.8 m^3 air during 24 h, this would lead to a nicotine intake of 2.2 (0.43-4.1) $\mu\text{g}/\text{kg}$ bw/day.

52. As noted in paragraph 45, an interim approach of using the study of Lindgren et al. (1999) to calculate a MOE is used here:

- Taking as the POD a value of 6.4 $\mu\text{g}/\text{kg}$ bw, from the LOAEL of 3.5 $\mu\text{g}/\text{kg}$ bw for HR effects in the Lindgren et al. (1999) study, as selected by EFSA (2009), with adjustment of 0.55 for bioavailability from i.v. to inhalation route:
 - i. For a 70 kg adult: the calculated nicotine exposure of 0.95 (0.2-1.8) $\mu\text{g}/\text{kg}$ bw/day would represent an MOE of 6.7 (3.5-32).
 - ii. For a 13.3 kg, 1-6 year-old child: the calculated nicotine exposure of 2.2 (0.4-4.1) $\mu\text{g}/\text{kg}$ bw/day would represent an MOE of 2.9 (1.6-16).
- Taking as the POD a value of 12.7 $\mu\text{g}/\text{kg}$ bw, from the NOAEL of 7 $\mu\text{g}/\text{kg}$ bw for EEG effects identified by COT, with adjustment of 0.55 for bioavailability from i.v. to inhalation route:
 - i. For a 70 kg adult: the calculated nicotine exposure of 0.95 (0.2-1.8) $\mu\text{g}/\text{kg}$ bw/day would represent an MOE of 13.4 (7.1-64).
 - ii. For a 13.3 kg, 1-6 year-old child: the calculated nicotine exposure of 2.2 (0.4-4.1) $\mu\text{g}/\text{kg}$ bw/day would represent an MOE of 5.8 (3.1-32).

53. In a 2006 review, 'The Health Consequences of Involuntary Exposure to Tobacco Smoke', published by the US Surgeon General, Chapter 4 reviewed 'Prevalence of Exposure to Second-hand Smoke', with a focus on measured concentrations of airborne nicotine (CDC 2006). This publication summarised data from numerous studies that had measured air nicotine levels in different settings where CC smoking was permitted, restricted, or banned, including homes, restaurants and bars, offices and other workplaces. Detailed information can be found in the report, at <https://www.ncbi.nlm.nih.gov/books/NBK44325/> (accessed

26/09/2019). In homes where CC smoking occurred, average nicotine levels were often in the range of 1-3 $\mu\text{g}/\text{m}^3$, with higher ranges measured during active smoking (e.g. 5-15 $\mu\text{g}/\text{m}^3$). Workplace studies showed a wide range of nicotine concentrations, with mean levels often in the range of 1-10 $\mu\text{g}/\text{m}^3$ but ranging up to around 50 $\mu\text{g}/\text{m}^3$ where smoking was allowed, and levels generally $< 1 \mu\text{g}/\text{m}^3$ where smoking was banned. In public places such as restaurants, bars, lounges, and other venues, nicotine levels ranged from less than detectable up to around 70 $\mu\text{g}/\text{m}^3$. A study of waiters exposed to second-hand smoke showed average nicotine levels of 5.8 $\mu\text{g}/\text{m}^3$, with an upper range of 68 $\mu\text{g}/\text{m}^3$, while a study in a cafeteria showed nicotine concentrations of 25-40 $\mu\text{g}/\text{m}^3$ in a smoking section, 2-5 $\mu\text{g}/\text{m}^3$ in a proximal non-smoking section, and $< 0.5 \mu\text{g}/\text{m}^3$ in a more distant non-smoking section. Average nicotine levels in bars and lounges were generally $>10 \mu\text{g}/\text{m}^3$ and often $>50 \mu\text{g}/\text{m}^3$, with maximum levels $>100 \mu\text{g}/\text{m}^3$ occasionally noted in bars (CDC 2006).

54. The mean nicotine level of 3.32 $\mu\text{g}/\text{m}^3$ measured by Czogala et al. (2014) (see Table 2), associated with use of an ENDS product by one user in a 39 m^3 chamber for two 5-min periods over 1 hour, is within the same range as that described in the U.S. Surgeon General report for levels of nicotine in households where CC smoking takes place.

55. Table 2 also lists two studies that measured nicotine levels in ambient air during indoor 'vaping events'. Johnson et al. (2018) reported a median nicotine level of 1.1 $\mu\text{g}/\text{m}^3$ (range <0.36 -2.2 $\mu\text{g}/\text{m}^3$) from measurements taken during four vaping events held in well-ventilated convention centres. However, Chen et al. (2017) measured a much higher mean ambient air nicotine concentration of 124.7 $\mu\text{g}/\text{m}^3$ during a vaping event held in a poorly ventilated venue. The mean air nicotine concentration of 124.7 $\mu\text{g}/\text{m}^3$ reported by Chen et al. (2017) is within the highest range of levels occasionally noted in bars where CC smoking was permitted, as described in the review of the U.S. Surgeon General.

Summary

56. Data are presented from clinical studies in which acute effects of nicotine exposure were evaluated in non-smokers. The studies included are limited to those in which the route of application was considered to be sufficiently representative of the kinetics of exposure to nicotine via inhalation of ENDS aerosol (i.v., s.c., intra-nasal, or inhalation). Studies using oral or dermal exposures were excluded. These data may be of use in providing a basis to calculate an HBGV for nicotine exposure from ENDS in bystanders.

57. A risk assessment is presented for exposure of ENDS users to nicotine. Taking a NOAEL of 7 $\mu\text{g}/\text{kg}$ bw for acute effects on EEG following i.v. nicotine exposure in CC smokers from the study of Lindgren et al. (1999), an HBGV of 1.3 $\mu\text{g}/\text{kg}$ bw/day was established for inhalation exposure to nicotine. Using data summarised in previous COT discussion papers on the highest reported mean nicotine level in ENDS aerosol and estimated average usage levels: a 15-puff ENDS-use session would expose the user to a nicotine level of approximately 7-fold

the COT HBGV; mean nicotine exposure during one day from an average ENDS use of 272-338 puffs would represent approximately 130- to 160-fold the COT HBGV. This would be equivalent to approximately 33-42% of the estimated average daily nicotine exposure from regular CC smoking.

58. As the Committee did not consider that the data of Lindgren et al. (1999) were suitable for calculation of an HBGV for nicotine exposure to non-smokers, consideration of bystander exposure to nicotine from ENDS is presented using an MOE approach. Using data from previous COT papers on highest mean nicotine concentrations in ambient air under experimental conditions, the MOE for mean daily exposure to nicotine for a 70 kg adult would be approximately 6.7 using a LOAEL of 3.5 µg/kg bw/day for HR effects, or 13.4 for a NOAEL of 7.0 µg/kg bw/day for EEG effects, based on the study of Lindgren et al. (1999). For a 13.3 kg, 1-6 year-old child, the respective MOE values would be 2.9 or 5.8. Mean nicotine concentrations measured in ambient air under experimental conditions of ENDS use were within the range noted in the U.S. Surgeon General report for background levels of nicotine in households where CC smoking takes place.

Questions for the Committee

59. Members are asked to consider the information provided in this paper and in particular:

- i. Do Members consider that it is appropriate to use numerical data from the studies that reported the highest mean levels of nicotine in ENDS aerosol or in ambient air in calculations of risk to users and bystanders, respectively?

Users

- ii. Do Members consider that a NOAEL of 7 µg/kg bw for EEG effects in the study of Lindgren et al. (1999) is the most suitable POD to calculate an HBGV for nicotine exposure in ENDS users? If not, what is the most appropriate POD?
- iii. Is it appropriate to establish a single HBGV covering acute and chronic exposure of users to nicotine from ENDS based on the acute effects on EEG noted in the study of Lindgren et al. (1999)?
- iv. Can the Committee draw any conclusions about (a) absolute and (b) relative risk for ENDS users from nicotine?

Bystanders

- v. Is an MOE approach using the Lindgren et al. (1999) data an appropriate method to use for consideration of bystander exposure to nicotine from ENDS? If so, is it preferable to base this on the POD of 3.5 µg/kg bw for heart-rate effects or 7 µg/kg bw for EEG effects?

This is a preliminary paper for discussion. It does not represent the views of the Committee and must not be quoted, cited or reproduced.

- vi. Alternately, do Members consider that any of the new data from clinical studies of acute cardiovascular and CNS effects of nicotine in non-smokers are suitable for use to calculate an HBGV for nicotine exposure to bystanders? If so, can the Committee identify a key study and point of departure to be used for this purpose?
- vii. Can the Committee draw any conclusions on the risks for bystanders from nicotine resulting from use of ENDS products?

Statement

- viii. 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
November 2019**

Abbreviations

| | |
|---------|---|
| ANCOVA | Analysis of co-variance |
| ANOVA | Analysis of variance |
| ARCI | Addiction Research Centre Inventory |
| BP | Blood pressure |
| CBF | Cerebral blood flow |
| CC | Conventional cigarette |
| CI | Confidence interval |
| CNS | Central nervous system |
| DBP | Diastolic blood pressure |
| E(N)NDS | Electronic nicotine (or non-nicotine) delivery system |
| ECG | Electrocardiogram |
| EEG | Electroencephalogram |
| ENDS | Electronic nicotine delivery system |
| ENNDS | Electronic non-nicotine delivery system |
| ESE | Early smoking experiences |
| FPV | Finger pulse volume |
| HR | Heart rate |
| LOAEL | Lowest observed adverse effect level |
| HBGV | Health-based guidance value |
| i.v. | Intravenous |
| MBG | Morphine Bazedrine Group |
| MOE | Margin of exposure |
| NNS | Nasal nicotine solution |
| NOAEL | No observed adverse effect level |
| NSE | Nicotine spray effects |
| PANAS | Positive And Negative Affect Scale |
| PCAG | Pentobarbital Chlorpromazine Alcohol Group |
| POMS | Profile of Mood States |
| rCBF | Regional cerebral blood flow |
| RR | Respiratory resistance |
| s.c. | Subcutaneous |
| SBP | Systolic blood pressure |
| SCL | Skin conductance level |
| SSS | Sensation seeking scale |
| UF | Uncertainty factor |
| VAS | Visual analogue scales |

References

- Ballbe, M., J. M. Martinez-Sanchez, X. Sureda, M. Fu, R. Perez-Ortuno, J. A. Pascual, E. Salto & E. Fernandez (2014) Cigarettes vs. e-cigarettes: Passive exposure at home measured by means of airborne marker and biomarkers. *Environ Res*, 135, 76-80.
- 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).
- Chen, R., A. Aherrera, C. Isichei, P. Olmedo, S. Jarmul, J. E. Cohen, A. Navas-Acien & A. M. Rule (2017) Assessment of indoor air quality at an electronic cigarette (Vaping) convention. *J Expo Sci Environ Epidemiol*.
- Czogala, J., M. L. Goniewicz, B. Fidelus, W. Zielinska-Danch, M. J. Travers & A. Sobczak (2014) Secondhand exposure to vapors from electronic cigarettes. *Nicotine Tob Res*, 16, 655-62.
- EFSA (2009) EFSA Statement. Potential risks for public health due to the presence of nicotine in wild mushrooms. *The EFSA Journal*, RN-286, 1-47.
- EPA (2008) Reregistration Eligibility Decision for Nicotine. List B. Case No. 2460. March 2008.
- Ettinger, U., S. C. R. Williams, D. Patel, T. M. Michel, A. Nwaigwe, A. Caceres, M. A. Mehta, A. P. Anilkumar & V. Kumari (2009) Effects of acute nicotine on brain function in healthy smokers and non-smokers: Estimation of inter-individual response heterogeneity. *NeuroImage*, 45, 549-561.
- Foulds, J., K. McSorley, J. Sneddon, C. Feyerabend, M. J. Jarvis & M. A. H. Russell (1994) Effect of subcutaneous nicotine injections on EEG alpha frequency in non-smokers: a placebo-controlled pilot study. *Psychopharmacology*, 115, 163-166.
- Foulds, J., J. A. Stapleton, N. Bell, J. Swettenham, M. J. Jarvis & M. A. H. Russell (1997) Mood and physiological effects of subcutaneous nicotine in smokers and never-smokers. *Drug and Alcohol Dependence*, 44, 105-115.
- Ghatan, P. H., M. Ingvar, L. Eriksson, S. Stone-Elander, M. Serrander, K. Ekberg & J. Wahren (1998) Cerebral effects of nicotine during cognition in smokers and non-smokers. *Psychopharmacology*, 136, 179-189.
- Hansson, L., N. B. Choudry, J. A. Karlsson & R. W. Fuller (1994) Inhaled nicotine in humans: effect on the respiratory and cardiovascular systems. *J Appl Physiol* (1985), 76, 2420-7.
- Johnson, J. M., L. P. Naeher, X. Yu, S. L. Rathbun, J. L. Muilenburg & J. S. Wang (2018) Air monitoring at large public electronic cigarette events. *International journal of hygiene and environmental health*.
- Laugesen, M. (2015) Nicotine and toxicant yield ratings of electronic cigarette brands in New Zealand. *N Z Med J*, 128, 77-82.
- Le Houezec, J., R. Halliday, N. L. Benowitz, E. Callaway, H. Naylor & K. Herzig (1994) A low dose of subcutaneous nicotine improves information processing in non-smokers. *Psychopharmacology*, 114, 628-634.

- Lindgren, M., L. Molander, C. Verbaan, E. Lunell & I. Rosen (1999) Electroencephalographic effects of intravenous nicotine--a dose-response study. *Psychopharmacology (Berl)*, 145, 342-50.
- Liu, J., Q. Liang, M. J. Oldham, A. A. Rostami, K. A. Wagner, I. G. Gillman, P. Patel, R. Savioz & M. Sarkar (2017) Determination of selected chemical levels in room air and on surfaces after the use of cartridge-and tank-based e-vapor products or conventional cigarettes. *International Journal of Environmental Research and Public Health*, 14.
- Maloney, J. C., M. K. Thompson, M. J. Oldham, C. L. Stiff, P. D. Lilly, G. J. Patskan, K. H. Shafer & M. A. Sarkar (2016) Insights from two industrial hygiene pilot e-cigarette passive vaping studies. *Journal of Occupational and Environmental Hygiene*, 13, 279-287.
- Melstrom, P., B. Koszowski, M. H. Thanner, E. Hoh, B. King, R. Bunnell & T. McAfee (2017) Measuring PM2.5, Ultrafine Particles, Nicotine Air and Wipe Samples Following the Use of Electronic Cigarettes. *Nicotine & Tobacco Research*, 19, 1055-1061.
- Myers, C. S., R. C. Taylor, B. J. Salmeron, A. J. Waters & S. J. Heishman (2013) Nicotine enhances alerting, but not executive, attention in smokers and nonsmokers. *Nicotine and Tobacco Research*, 15, 277-281.
- O'Connell, G., S. Colard, X. Cahours & J. D. Pritchard (2015) An Assessment of Indoor Air Quality before, during and after Unrestricted Use of E-Cigarettes in a Small Room. *Int J Environ Res Public Health*, 12, 4889-907.
- Perkins, K. A., D. Gerlach, M. Broge, J. E. Grobe, M. Sanders, C. Fonte, J. Vender, C. Cherry & A. Wilson (2001) Dissociation of nicotine tolerance from tobacco dependence in humans. *Journal of Pharmacology and Experimental Therapeutics*, 296, 849-856.
- Perkins, K. A., D. Gerlach, M. Broge, J. E. Grobe & A. Wilson (2000) Greater sensitivity to subjective effects of nicotine in nonsmokers high in sensation seeking. *Experimental and Clinical Psychopharmacology*, 8, 462-471.
- Perkins, K. A., J. E. Grobe, C. Fonte, J. Goettler, A. R. Caggiula, W. A. Reynolds, R. L. Stiller, A. Scierka & R. G. Jacob (1994) Chronic and acute tolerance to subjective, behavioral and cardiovascular effects of nicotine in humans. *Journal of Pharmacology and Experimental Therapeutics*, 270, 628-638.
- Perkins, K. A., C. Lerman, S. Coddington, C. Jetton, J. L. Karelitz, A. Wilson, J. R. Jennings, R. Ferrell, A. W. Bergen & N. L. Benowitz (2008b) Gene and gene by sex associations with initial sensitivity to nicotine in nonsmokers. *Behavioural Pharmacology*, 19, 630-640.
- Perkins, K. A., C. Lerman, S. Coddington & J. L. Karelitz (2008a) Association of retrospective early smoking experiences with prospective sensitivity to nicotine via nasal spray in nonsmokers. *Nicotine and Tobacco Research*, 10, 1335-1345.
- Postma, P., J. A. Gray, T. Sharma, M. Geyer, R. Mehrotra, M. Das, E. Zachariah, M. Hines, S. C. R. Williams & V. Kumari (2006) A behavioural and functional neuroimaging investigation into the effects of nicotine on sensorimotor gating

in healthy subjects and persons with schizophrenia. *Psychopharmacology*, 184, 589-599.

- Russell, M. A. H., M. J. Jarvis, G. Jones & C. Feyerabend (1990) Non-smokers show acute tolerance to subcutaneous nicotine. *Psychopharmacology*, 102, 56-58.
- Saffari, A., N. Daher, A. Ruprecht, C. De Marco, P. Pozzi, R. Boffi, S. H. Hamad, M. M. Shafer, J. J. Schauer, D. Westerdahl & C. Sioutas (2014) Particulate metals and organic compounds from electronic and tobacco-containing cigarettes: comparison of emission rates and secondhand exposure. *Environ Sci Process Impacts*, 16, 2259-67.
- Schober, W., L. Fembacher, A. Frenzen & H. Fromme (2019) Passive exposure to pollutants from conventional cigarettes and new electronic smoking devices (IQOS, e-cigarette) in passenger cars. *Int J Hyg Environ Health*.
- Schober, W., K. Szendrei, W. Matzen, H. Osiander-Fuchs, D. Heitmann, T. Schettgen, R. A. Jorres & H. Fromme (2014) Use of electronic cigarettes (e-cigarettes) impairs indoor air quality and increases FeNO levels of e-cigarette consumers. *Int J Hyg Environ Health*, 217, 628-37.
- Soria, R., J. M. Stapleton, S. F. Gilson, A. Sampson-Cone, J. E. Henningfield & E. D. London (1996) Subjective and cardiovascular effects of intravenous nicotine in smokers and non-smokers. *Psychopharmacology (Berl)*, 128, 221-6.
- Swan, G. E., C. N. Lessov-Schlaggar, R. E. Krasnow, K. C. Wilhelmsen, P. Jacob Iii & N. L. Benowitz (2007) Genetic and environmental sources of variation in heart rate response to infused nicotine in twins. *Cancer Epidemiology Biomarkers and Prevention*, 16, 1057-1064.
- UK-DAR. 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.
- West, R. J. & M. J. Jarvis (1986) Effects of nicotine on finger tapping rate in non-smokers. *Pharmacology, Biochemistry and Behavior*, 25, 727-731.

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 from September 2019 COT meeting: updated risk assessments for nicotine exposure from ENDS.

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

The following literature searches were performed by NCET at WRc/IEH-C under contract to PHE on 14/20/2019 in Scopus and PubMed.

SCOPUS

(((TITLE (nicotine) AND NOT TITLE-ABS-KEY (tablet OR gum OR patch OR ingest*))) AND ((TITLE-ABS-KEY ("non smoker*" OR "non-smoker*" OR nonsmoker*) AND TITLE-ABS-KEY (cardio* OR "heart rate" OR "blood pressure" OR "central nervous system" OR brain OR "spinal cord" OR electrocardiogram OR electroencephalogram OR ecg OR eeg)))) AND ((KEY (human OR humans) AND NOT KEY (animal OR animals))) AND (EXCLUDE (SUBJAREA , "AGRI") OR EXCLUDE (SUBJAREA , "ENVI") OR EXCLUDE (SUBJAREA , "SOCI") OR EXCLUDE (SUBJAREA , "ARTS") OR EXCLUDE (SUBJAREA , "MULT") OR EXCLUDE (SUBJAREA , "COMP") OR EXCLUDE (SUBJAREA , "ENGI") OR EXCLUDE (SUBJAREA , "CENG") OR EXCLUDE (SUBJAREA , "CHEM") OR EXCLUDE (SUBJAREA , "MATH") OR EXCLUDE (SUBJAREA , "DECI") OR EXCLUDE (SUBJAREA , "DENT") OR EXCLUDE (SUBJAREA , "MATE")) AND (LIMIT-TO (LANGUAGE , "English") OR EXCLUDE (LANGUAGE , "Portuguese") OR EXCLUDE (LANGUAGE , "German") OR EXCLUDE (LANGUAGE , "Turkish"))): 97.

PubMed

(((((nicotine[Title]) NOT (tablet[Title/Abstract] OR gum[Title/Abstract] OR patch[Title/Abstract] OR ingest*[Title/Abstract]))) AND (((non smoker*[Title/Abstract] OR "non-smoker*[Title/Abstract] OR nonsmoker*[Title/Abstract])) AND (cardio*[Title/Abstract] OR "heart rate"[Title/Abstract] OR "blood pressure"[Title/Abstract] OR "central nervous system"[Title/Abstract] OR brain[Title/Abstract] OR "spinal cord"[Title/Abstract] OR eeg[Title/Abstract] OR ecg[Title/Abstract] OR electrocardiogram[Title/Abstract] OR electroencephalogram[Title/Abstract]))) AND english[Language]) AND Humans[Mesh]) AND (((clinical study"[Publication Type]) OR "clinical trial"[Publication Type]) AND Humans[Mesh]): 29.