TOX/2019/46

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

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Follow up to Paper 12: An overview of strategies to reduce nicotine addiction using low-nicotine-content products.

Background

1. As part of the review on the potential toxicity of electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (collectively abbreviated to E(N)NDS), the COT has been reviewing potential toxicity of exposure to nicotine from these products. At the July 2019 COT meeting, a review of toxicological data on nicotine was discussed (TOX/2019/38). As a follow-up to this discussion, the Committee requested that information be provided on reduced nicotine content cigarettes, a product that is being developed with the aim to reduce population levels of addiction to nicotine and of cigarette smoking. This current paper presents a brief overview of the development of this field in the U.S.

Search scope

2. A brief search of PubMed was performed on 29/07/2019 and 5 review articles and/or commentaries were selected to provide a summary overview of the field. In addition, a description of the key initial publication by Benowitz and Henningfield (1994) is provided.

Benowitz NL, Henningfield JE (1994). Establishing a nicotine threshold for addiction. The implications for tobacco regulation. N Engl J Med, 331, 123–125.

3. The report by Benowitz & Henningfield (1994) is considered to be the key initial publication in proposing the concept of reducing nicotine levels in tobacco cigarettes below the level of addictivity, with the aim to reduce tobacco-related harm at the population level. The paper addresses the following aspects.

Is there a threshold level of nicotine intake associated with addiction?

4. Addiction is defined as the compulsive use of a drug that has psychoactivity and that may be associated with tolerance and physical dependence (including withdrawal upon cessation). For smokers, addiction is assumed to involve daily smoking, difficulty not smoking daily, and high likelihood of withdrawal symptoms on cessation. However, it is noted that approximately 10% of Americans are tobacco ‘chippers’, meaning people who regularly smoke ≤ 5 cigarettes/day, do not appear to be addicted, do not have withdrawal symptoms on stopping, and can skip a day or stop smoking without distress. Benowitz and Henningfield (1994) used published data from addicted daily smokers and smokers of ≤ 5 cigarettes per day to calculate

a proposed threshold for nicotine that can readily establish and maintain addiction, as follows:

- The average blood cotinine in addicted smoker is 300 ng/mL.
- The average serum cotinine in smokers of ≤ 5 cigarettes per day is 54 ng/mL, and average cigarette consumption in this group is 3.9 per day. The cotinine level normalised to 5 cigarettes per day would be 70 ng/mL.
- From these data, a cut-off threshold of 50–70 ng/mL cotinine can be estimated for addictive threshold.
- Studies of nicotine and cotinine infusion indicate that daily nicotine intake can be calculated as 0.08 times blood cotinine level, hence 50–70 ng/mL cotinine would correspond to an intake of 4–6 mg per day nicotine.
- A threshold level that can readily establish and sustain addiction is proposed as 5 mg nicotine per day.

Nicotine delivery from cigarettes

5. An average cigarette contains 8–9 mg nicotine[1]. The concentration of nicotine in tobacco is 1.5–2.5%. A cigarette typically delivers around 1 mg nicotine (range approximately 0.3–3.2 mg) to the circulation of a smoker (bioavailability 12%, range approximately 3–40%).

6. Machine-determined yields correlate poorly with daily nicotine intake evaluated from human smokers. Reducing the availability of cigarettes from 38 per day to 5 per day increased nicotine intake per cigarette by approximately 3-fold, consistent with a maximum absolute bioavailability of 40%. If cigarettes were redesigned, bioavailability would have to be reassessed in people smoking the redesigned cigarettes (i.e. not via machine smoking).

Threshold levels of nicotine in cigarettes as a way to avert addiction

7. The absolute level of nicotine in a cigarette could be regulated to limit the maximal obtainable dose.

8. The authors assumed an estimated target nicotine dose of ≤ 5 mg/day to avert addiction, and that a young person may smoke up to 30 cigarettes per day, giving a maximal available (systemic) dose of 0.17 mg nicotine per cigarette as the threshold level for a less-addictive cigarette. Assuming maximum bioavailability of 40% with intensive smoking, the authors suggested an absolute limit of 0.4–0.5 mg nicotine per cigarette should be adequate to prevent or limit the development of addiction in most young people. This may, nevertheless, be sufficient to provide enough nicotine for taste and sensory stimulation.

[1] As reported in Benowitz & Henningfield (1994). More recent publications report a level of 10–15 mg/cigarette.

Possible strategy for regulation

9. At the time of the publication, Benowitz & Henningfield (1994) proposed a strategy to minimise hardship to current addicted adult smokers, whereby the level of nicotine in tobacco could be reduced gradually over perhaps 10–15 years. The intention would be that cigarettes could still be sold, but the number of addicted smokers would be substantially reduced. In the absence of addiction, levels of tobacco consumption, and the associated tobacco-related illness, would fall sharply.

10. Points that were noted as requiring consideration in this strategy included the choice of a theoretical value for threshold for addiction, the need to demonstrate that restricting levels of nicotine would actually prevent addiction, and concerns that currently addicted smokers would engage in more intense compensatory smoking.

11. The alternative concept of reducing tobacco harm through nicotine-enriched cigarettes was also commented, the theory being that the need to smoke fewer cigarettes to obtain the same nicotine dose would lead to lower exposure to cigarette smoke-related toxins. However, this was considered to be of likely minimal use on a personal level, given that cigarette smoke is highly toxic at even small doses, and not useful on a population level where the goal would be the prevention of nicotine addiction and reduction in the prevalence of cigarette smoking, and ultimately the elimination of exposure to toxins in tobacco smoke

A summary of information from 2 review articles by Benowitz and Henningfield (Benowitz and Henningfield 2013, Benowitz and Henningfield 2018)

12. As described in paragraphs 3-11, above, Benowitz and Henningfield (1994) initially proposed the reduction of nicotine content in cigarettes to non-addictive levels as a strategy to reduce the risk that future generations would become addicted to smoking cigarettes. This was based on the theory that in the absence of nicotine addiction, individuals would be far less likely to transition from experimental smoking (which usually takes place in a social context during childhood or adolescence) to a situation of addicted, regular smoking to obtain pharmacological effects. Authors estimated that a threshold of 0.4 mg nicotine/cigarette rod (‘very low nicotine content cigarettes’) would result in a minimally addictive cigarette. A conventional cigarette contains 10–15 mg nicotine. The review by Benowitz and Henningfield (2018) noted that this concept has subsequently been supported by the study of Donny et al. (2015) (see paragraph 14), which demonstrated reduced dependence and fewer cigarettes smoked per day associated with use of cigarettes containing 0.4 mg nicotine/rod. Nevertheless, other analyses have suggested that the threshold for addiction may be lower than 0.4 mg nicotine/rod (Hatsukami et al. 2010b, Sofuoglu and LeSage 2012) (see later section describing the reviews by Sofuoglu & LeSage 2012 and Jensen et al. 2016).

13. Since the initial publication by Benowitz and Henningfield (1994), substantial research has been carried out and clinical trials have demonstrated that nicotine reduction is feasible. Benowitz et al. (2007) carried out a study in which 20 smokers

smoked their usual brand cigarette for 1 week, then changed, weekly, to 5 progressively lower nicotine content cigarettes for 5 subsequent weeks (from 10 mg down to 0.6 mg per cigarette). Plasma cotinine and urinary tobacco-specific nitrosamine (TSNA) marker decreased over time, while the number of cigarettes smoked per day, and biomarkers of smoke exposure did not change significantly during the study. A subsequent, larger study using a monthly nicotine-reduction protocol, and including a group who continued to smoke own-brand cigarettes, produced similar findings (Benowitz et al. 2012). In both studies, some smokers quit smoking spontaneously, and those continuing to smoke reported lower dependence.

14. A study by Hatsukami et al. (2010a) compared switching directly from users own cigarettes to either reduced-nicotine cigarettes (0.05 mg yield) or to nicotine lozenges (0.3 mg yield) for 6 weeks, with no nicotine dose tapering. The reduced-nicotine cigarette group had reduced indicators of exposure to nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), without increased cigarette consumption, and showed higher rates of smoking cessation compared with the lozenge group. Benowitz and Henningfield (2013) interpreted these findings to suggest that the nicotine content of cigarettes might be able to be reduced quickly rather than gradually over years. In a study reported by Donny et al. (2015), 780 participants were randomised to use cigarettes containing 15.8, 5.2, 2.4, 1.3, or 0.4 mg nicotine per gram tobacco[2] or to usual brand cigarettes for 6 weeks. Participants assigned to cigarettes with 2.4, 1.3, or 0.4 mg nicotine/g tobacco smoked fewer cigarettes than the 15.8 mg/g or own-brand smokers. Cigarettes with lower nicotine content were associated with reduced exposure to and dependence on nicotine and reduced craving during abstinence from smoking, without significantly increasing the expired carbon monoxide level or total puff volume, suggesting minimal compensation through increased number of cigarettes smoked.

15. In 2009, the U.S. ‘Family Smoking Prevention and Tobacco Control Act’ (Tobacco Control Act) authorised the U.S. Food and Drug Administration (FDA) to reduce nicotine to non-addictive levels (although not to zero) (FDA 2009), at which point research was instigated to gather knowledge on which to base future decisions for implementation. In 2016, FDA authority was extended to include regulation of alternative nicotine delivery systems (ANDS) and other non-cigarette tobacco products (FDA 2016). The subsequent 2017 FDA plan for tobacco regulation focussed on nicotine, including the intent to develop a rule to reduce nicotine to non-addictive levels while promoting conditions to enable implementation (Gottlieb and Zeller 2017, cited in Benowitz & Henningfield 2018), including the availability of medicinal and other non-combustible nicotine products to facilitate transition from cigarette smoking. An ‘Advance Notice of Proposed Rulemaking’ was issued in 2018 to obtain public input on the plan to reduce nicotine levels in cigarettes to non-addictive levels. The hope is that this will prevent or substantially diminish the acquisition of addicted smoking in youth and, in addition, prompt the majority of

[2] Based on data obtained from an internet search, 1 cigarette rod generally appears to contain around 1 g tobacco.

addicted smokers to quit. In summary of their review article, Benowitz and Henningfield (2018) commented that “A nicotine-focused regulatory framework could have a tremendously beneficial effect on public health. Reducing the addictiveness of cigarettes by nicotine reduction would prevent or markedly diminish the acquisition of addicted smoking in youth, and would most likely prompt many or most addicted smokers to quit smoking. The availability of acceptable and less hazardous forms of nicotine would provide support and enhance acceptability of nicotine reduction in tobacco.”

A summary of information from a review article by Bevins and colleagues (Bevins et al. 2018)

16. The review by Bevins et al (2018) discusses nicotine reduction, including reduced nicotine content cigarettes, from the point of view of behavioural pharmacology.

17. Diagnostic tools for addiction are weak predictors of actual rates of tobacco use. A proposed (better) alternative to use of the addiction threshold is the reinforcement threshold, defined as the minimum dose required to control an increase or maintenance of nicotine self-administration behaviour. Reinforcement is assumed to be a contributor to addiction, and a nicotine dose below the reinforcement threshold should be below the threshold for addiction and dependence.

18. Studies in rats have suggested that the reinforcement threshold is in the range of 3–10 µg/kg bw. A study by Grebenstein et al. (2013), in which nicotine was given intravenously at gradually decreasing dose, showed that a dose as low as 3.2 µg/kg bw led to maintained lever pressing at levels higher than saline, with no sex-difference in the reinforcement threshold.

19. Other thresholds that can be considered include discrimination threshold, and reinforcer-enhancement threshold. In humans, the nicotine discrimination threshold was reported to be around 11 mg nicotine per gram tobacco, but with wide inter-individual variation (Perkins et al. 2016). A review by Smith et al. (2017) is noted as being a useful overview of this area.

20. The review of Bevins et al. (2018) notes that although policies regarding nicotine reduction will be targeted at the population level, the concept of individual differences in susceptibility to nicotine is also very important. If, for example, thresholds are set at a ‘population average’, then approximately half of the population will be missed or differently impacted. Individuals with psychiatric disorders (e.g. schizophrenia, depression) or other comorbid risk factors (alcohol use disorder, chronic stress) are also highlighted as having potentially enhanced vulnerabilities.

A summary of information from 2 review articles by Sofuoglu and colleagues (Sofuoglu and LeSage 2012, Jensen, DeVito and Sofuoglu 2016a)

21. The two review articles by Sofuoglu and colleagues discuss nicotine pharmacology and reinforcement, and the use of intravenous (i.v.) nicotine delivery as an appropriate method to study reinforcement in humans.

22. In order for reduced nicotine content cigarettes to be successful, it is first necessary to determine a threshold dose for the addictive effects of nicotine, and then to characterise individual differences in the addictive threshold, noting that this may vary with age, gender differences, and presence of comorbid mental health problems.

23. Three specific concepts of relevance are noted:

- abuse potential – the intrinsic pharmacological effect of a drug that is reported as 'liked' by users or induces positive subjective effects (e.g. drug liking, good drug effects, high, want more drugs) that are concomitant with behavioural reinforcement (self-administration)

- addiction threshold – the minimum nicotine intake required to initiate or maintain behaviours that meet a clinical criterion for addiction (e.g. DSM 5 criteria for tobacco use disorder or Fagerström Test for Nicotine Dependence (FTND) score ≥ 5.

- reinforcement threshold – the lowest nicotine dose that will initiate or maintain self-administration behaviours (tobacco-product use) (Hatsukami et al. 2010b).

24. The addiction threshold is a difficult concept to measure due to a lack of consensus on criteria for nicotine addiction and a weak relationship between nicotine addiction and actual rates of tobacco use. However, reinforcement threshold is easier to examine using established pharmacological methods, and this threshold is one of the critical steps in determining the addiction threshold for nicotine in tobacco products.

25. Support for addictive effects of nicotine has been demonstrated in animal models and human clinical studies (Henningfield et al 1983, Rose & Corrigall 1997, Corrigall et al 2000, Donny et al 2000, Fattorre et al 2002, Harvey et al 2004, LeFoll et al 2007, Sofuoglu et al 2008, Mello et al 2013, Goodwin et al 2015, refs cited in Jensen et al 2016a) and in studies on neurobiological effects of nicotine on reward pathways in the brain (Corrigall et al 1992, 1994, Pontieri et al 1996, Pidoplichko et al 1997, refs cited in Jensen et al 2016a).

26. Genetic variation has been shown, associated with differences in genes encoding nicotinic acetylcholine receptors (nAChRs) and nicotine-metabolising enzymes (primarily CYP2A6). nAChRs comprise combinations of subunits (α2–α10

and β2–β4), with differences in agonist affinity and desensitisation thresholds based on subunit composition. Aversive effects to high doses of nicotine are partly mediated through α5-containing nAChRs, and reinforcing effects of high doses have been shown in α5-knockout mice (Fowler & Kenny, cited in Jensen et al 2016), while genetic variation at this locus has been associated with heavy cigarette use in humans. It is suggested that aversion to high doses of nicotine may play a role in low likelihood of uptake or becoming a regular daily smoker (Hu et al 2006, Sartor et al 2010, refs cited in Jensen et al 2016a).

27. Regarding animal data, it is presently unclear how these may translate to regulatory policy for tobacco control in humans. For human studies, the challenge has been to establish suitable systems for precise and reproducible dosing in order to investigate dose-dependent effects of nicotine on reinforcement. Jensen et al. (2016a) review different options, including ENDS, nicotine replacement therapies (NRT) (gum, lozenge, nasal spray), and i.v. administration.

28. ENDS are noted to deliver nicotine slower than cigarette smoking but faster than most other products, with rates varying greatly depending both on the ENDS product and the individual user characteristics. For the majority of users, absorption is slower and mean peak blood nicotine is lower than that achieved by smoking. Delivery of precise doses of nicotine cannot be reliably controlled in an experimental setting and ENDS are not considered to be optimal tools for examining dose-dependent effects of nicotine on reinforcement. In addition, as with cigarettes, the co-presence of sensory stimuli paired with nicotine in ENDS (taste, smell, visual) makes evaluation of primary reinforcing effects more difficult.

29. NRT products produce peak plasma nicotine approximately 10-fold slower compared with tobacco cigarette smoking. In experimental human studies, the products generally do not elicit ‘drug liking’ responses indicative of reinforcement, and in some cases provoke aversive effects. They are not associated with strong positive reinforcing effects.

30. Precise, reproducible dosing of nicotine can be best obtained using i.v. infusion, using nicotine diluted in saline solution, and the ‘gold standard’ behavioural measure for nicotine reinforcement is i.v. self-administration at levels greater than saline. Administration can be modulated to represent kinetics of intake from tobacco smoking. Studies have demonstrated that i.v. infusion can produce arterial and venous plasma nicotine concentrations that are similar to those occurring via smoking, with rapid time to peak concentration (20 s for smoking vs. 30 s for i.v. infusion) (Rose et al 1999, cited in Jensen et al 2016a). Studies have shown reinforcing effects of i.v. nicotine by self-administration in adult smokers (Henningfield et al 1983, Harvey et al 2004, Sofluogu et al 2008, Mello et al 2013, Goodwin et al 2015, refs cited in Jensen et al 2016a), but few studies have evaluated the dose-response curve for reinforcement in humans.

31. Studies have shown some sex differences in nicotine-related reinforcement responses. A randomised, double-blind study investigated dose-dependent effects

on reinforcement within a limited range of 'low to moderate' doses (0.1, 0.2, 0.3, 0.4 mg nicotine, considered to represent 1–4 puffs of a tobacco cigarette) (Jensen et al. 2016b). After random-order infusion of nicotine and of saline, tobacco smokers (14 female, 12 male) were given 6 force-trials in which they chose (still blinded) an i.v. infusion of either saline or the nicotine solution. In males, there was a negative linear relationship between choice and nicotine dose (higher choice at 0.1 and 0.2 mg than at 0.4 mg), while no variation of choice over dose was seen for females. There was no overall choice preference over saline at any condition. All nicotine doses were rated as more pleasurable than saline, which authors considered indicated abuse potential at all doses. Previous studies have also indicated less nicotine dose sensitivity in females than males. Effects related to menstrual cycle are also discussed.

32. Individual genetic effects were also tested using an i.v. nicotine infusion protocol. A group of daily smokers, with or without a common functional SNP in the nAChR α5 subunit gene, which is linked to heavy smoking, received infusions of saline, 0.5 and 1.0 mg/70 kg bw nicotine (equivalent to approximately 1–1.5 tobacco cigarettes) over 60 s. The heavy-smoking-allele group showed lower ratings of subjective aversive effects over time, while responses for pleasurable and stimulatory subjective effects were not different (Jensen et al. 2015).

Summary

33. Some recent review articles are summarised which address the topic of reduced nicotine content cigarettes. The concept of this 'very low nicotine content' strategy is to make cigarettes non-addictive, so that smokers and novice smokers do not transition from experimental or occasional smoking to addiction (Benowitz and Henningfield 2013).

34. Nicotine reduction as a strategy assumes a threshold for nicotine exposure that is necessary to produce reinforcing effects and to sustain addiction. This threshold is likely to vary between individuals. Initial calculations estimated that reducing total nicotine content in 1 cigarette rod to 0.5 mg would minimise the addictiveness of cigarettes (Benowitz and Henningfield 1994). However, subsequent analysis has suggested that this threshold may be lower (Hatsukami et al. 2010b, Sofuoglu and LeSage 2012).

35. Measuring nicotine addictiveness is not straightforward and there is not yet a clear consensus on distinguishing nondependent and dependent smokers (Sofuoglu and LeSage 2012). Commonly used tools include FTND scale and DSM-IV criteria. An alternative concept is to measure the reinforcement threshold (Hatsukami et al. 2010b), which may be defined as the lowest nicotine dose that will increase or maintain nicotine self-administration behaviour. Because dependence does not occur if the substance is not reinforcing, this threshold is likely to be below the addiction threshold. The reinforcement threshold can be measured using short-term studies of self-administration, both in human and animals (Sofuoglu and LeSage 2012)

36. Clinical studies have indicated that smokers do not substantially increase their smoking rates (amount of daily smoke exposure) to compensate when switching to cigarettes with very low nicotine content (Benowitz et al. 2007, Hatsukami et al. 2010a, Benowitz et al. 2012, Donny et al. 2015).

Questions for the Committee

37. Members are asked to consider the paper and in particular:

i. Do Members consider that data on reduced nicotine content cigarettes may be of use in evaluating threshold levels for effects of addiction in users or bystanders exposed to nicotine from ENDS?

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat
September 2019

Abbreviations

ANDS	Alternative nicotine delivery systems
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
FTND	Fagerström Test for Nicotine Dependence
i.v.	Intravenous
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NRT	Nicotine replacement therapy
U.S. FDA	United States Food and Drug Administration
TSNA	Tobacco-specific nitrosamine(s)

References

Benowitz, N. L., K. M. Dains, S. M. Hall, S. Stewart, M. Wilson, D. Dempsey & P. Jacob, 3rd (2012) Smoking behavior and exposure to tobacco toxicants during 6 months of smoking progressively reduced nicotine content cigarettes. Cancer Epidemiol Biomarkers Prev, 21**,** 761-9.

Benowitz, N. L., S. M. Hall, S. Stewart, M. Wilson, D. Dempsey & P. Jacob, 3rd (2007) Nicotine and carcinogen exposure with smoking of progressively reduced nicotine content cigarette. Cancer Epidemiol Biomarkers Prev, 16**,** 2479-85.

Benowitz, N. L. & J. E. Henningfield (1994) Establishing a nicotine threshold for addiction. The implications for tobacco regulation. N Engl J Med, 331**,** 123-5.

Benowitz, N.L. & J.E. Henningfield (2013) Reducing the nicotine content to make cigarettes less addictive. Tob Control, 22 Suppl 1**,** i14-7.

Benowitz, N.L. & J.E. Henningfield (2018) Nicotine Reduction Strategy: State of the science and challenges to tobacco control policy and FDA tobacco product regulation. Prev Med, 117**,** 5-7.

Bevins, R. A., S. T. Barrett, Y. W. Huynh, B. M. Thompson, D. A. Kwan & J. E. Murray (2018) Experimental analysis of behavior and tobacco regulatory research on nicotine reduction. J Exp Anal Behav, 110**,** 1-10.

Donny, E. C., R. L. Denlinger, J. W. Tidey, J. S. Koopmeiners, N. L. Benowitz, R. G. Vandrey, M. al'Absi, S. G. Carmella, P. M. Cinciripini, S. S. Dermody, D. J. Drobes, S. S. Hecht, J. Jensen, T. Lane, C. T. Le, F. J. McClernon, I. D. Montoya, S. E. Murphy, J. D. Robinson, M. L. Stitzer, A. A. Strasser, H. Tindle & D. K. Hatsukami (2015) Randomized Trial of Reduced-Nicotine Standards for Cigarettes. N Engl J Med, 373**,** 1340-9.

Grebenstein, P., D. Burroughs, Y. Zhang & M. G. LeSage (2013) Sex differences in nicotine self-administration in rats during progressive unit dose reduction: implications for nicotine regulation policy. Pharmacol Biochem Behav, 114-115**,** 70-81.

Hatsukami, D. K., M. Kotlyar, L. A. Hertsgaard, Y. Zhang, S. G. Carmella, J. A. Jensen, S. S. Allen, P. G. Shields, S. E. Murphy, I. Stepanov & S. S. Hecht (2010a) Reduced nicotine content cigarettes: effects on toxicant exposure, dependence and cessation. Addiction, 105**,** 343-55.

Hatsukami, D. K., K. A. Perkins, M. G. Lesage, D. L. Ashley, J. E. Henningfield, N. L. Benowitz, C. L. Backinger & M. Zeller (2010b) Nicotine reduction revisited: science and future directions. Tob Control, 19**,** e1-10.

Jensen, K. P., E. E. DeVito, A. I. Herman, G. W. Valentine, J. Gelernter & M. Sofuoglu (2015) A CHRNA5 Smoking Risk Variant Decreases the Aversive Effects of Nicotine in Humans. Neuropsychopharmacology, 40**,** 2813-21.

Jensen, K. P., E. E. DeVito & M. Sofuoglu (2016a) How Intravenous Nicotine Administration in Smokers Can Inform Tobacco Regulatory Science. Tob Regul Sci, 2**,** 452-463.

Jensen, K. P., E. E. DeVito, G. Valentine, R. Gueorguieva & M. Sofuoglu (2016b) Intravenous Nicotine Self-Administration in Smokers: Dose-Response Function and Sex Differences. Neuropsychopharmacology, 41**,** 2034-40.

Perkins, K. A., N. Kunkle, J. L. Karelitz, V. C. Michael & E. C. Donny (2016) Threshold dose for discrimination of nicotine via cigarette smoking. Psychopharmacology (Berl), 233**,** 2309-17.

Smith, T. T., L. E. Rupprecht, R. L. Denlinger-Apte, J. J. Weeks, R. S. Panas, E. C. Donny & A. F. Sved (2017) Animal Research on Nicotine Reduction: Current Evidence and Research Gaps. Nicotine Tob Res, 19**,** 1005-1015.

Sofuoglu, M. & M. G. LeSage (2012) The reinforcement threshold for nicotine as a target for tobacco control. Drug Alcohol Depend, 125**,** 1-7.