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TOX/202020/59

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

A summary of data on the presence and pharmacokinetics of nicotine salts in electronic nicotine delivery systems (ENDS) products.

Introduction

1. The nicotine present in electronic nicotine delivery system (ENDS) products has standardly been the 'freebase' form, in an e-liquid comprising a base of propylene glycol (PG) and/or glycerol. Freebase nicotine is volatile, with a tendency to deposit in the mouth and upper respiratory tract. Some more recently marketed products contain nicotine in the form of a salt, owing to the inclusion in the e-liquid of an organic acid, for example benzoic acid or lactic acid. The acid lowers the pH of the e-liquid, leading to a shift of nicotine towards the protonated rather than freebase form. Protonated nicotine is reported to be less harsh and bitter on inhalation than freebase nicotine and hence is less irritating to the throat and lungs. Nicotine salt is less volatile than freebase nicotine and thus a higher proportion of inhaled nicotine reaches the lungs.

2. The Committee is asked to consider the effects of use of nicotine salts in ENDS products on internal exposure to nicotine.

3. This paper provides a short overview of publicly available information of relevance to the presence of nicotine salts in ENDS products and the pharmacokinetics of nicotine when inhaled in the salt form from ENDS products. Some commentary on the historical development of traditional combustible tobacco products with relation to modulation of smoke pH and nicotine form is also included.

Search strategy

4. Searches of Scopus and PubMed for publications relating to 'nicotine salts' and 'electronic cigarettes/ENDS' were conducted on 05/06/2020, and the search of PubMed was briefly updated on 24/08/2020. Further searches, using the PubMed and Scifinder databases, were conducted on 09/10/2020, using a broader range of search terms including combinations of the following terms: nicotine, tobacco, cigarette, pH, salt, nicotine salt, bioavailability, inhalation. Searches of 'grey literature' were also conducted. Approximately 500 citations were identified. A final set of 46 publications was catalogued for the present report, of which 16 are directly cited here.

5. Full details of searches are provided at Annex A.

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Background information

Hazard classification of nicotine and nicotine salts under the CLP Regulation (provided by HSE)

6. Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP) requires suppliers of chemical substances or mixtures to identify their hazards (dangers) before placing them on the market, a process is known as 'classification'. Chemicals that are classified as hazardous must then be labelled and packaged according to CLP requirements. This is to ensure people using them – either at work or as consumers – can understand any hazardous effects they could have on human health or the environment and how to protect against that harm. Some chemicals have 'harmonised' classification and labelling which means that suppliers must use them when classifying their products.

7. E-liquids are 'mixtures' within the meaning of CLP and therefore suppliers of e-liquids need to consider how the requirements of CLP apply to their products. An e-liquid mixture might contain a number of substances that are individually classified as hazardous under CLP – and not just nicotine (or a salt of nicotine). Many flavourings used in e-liquid products are hazardous in their own right, e.g. as skin sensitisers or skin/eye irritants. Suppliers must consider each constituent substance to derive a suitable hazard classification for the overall mixture. While suppliers can use data on the mixture as a whole, more often than not such data are lacking, and suppliers will instead base their classification on a calculation approach that assumes that the higher the concentration of a hazardous substance in a mixture, the more likely it is the mixture will present the same hazards.

8. **Nicotine** has harmonised classification and labelling under CLP as:

- Acute Tox. 2; H300 – Fatal if swallowed
- Acute Tox. 2; H310 – Fatal in contact with skin
- Acute Tox. 2; H330 – Fatal in inhaled
- Aquatic Chronic 2; H411 – Toxic to aquatic life with long lasting effects

The harmonised classification for nicotine also sets Acute Toxicity Estimates (ATE); these are numerical values that must be used to calculate the classification for acute toxicity of a mixture which contains a substance classified for that hazard as one of its components. Table 1 below indicates the CLP hazard classification that might be required for mixtures containing different concentrations of nicotine. However, some suppliers may apply classifications that vary from those below and there might be legitimate reasons for doing so, e.g. if they adjust for relative density.

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Table 1. CLP hazard classifications for mixtures containing nicotine (source HSE)

Nicotine	**Exposure route**		
Hazard category	**Oral**	**Dermal**	**Inhalation (dusts/mists)**
Acute Tox 1 (Fatal if…)	N/A	N/A	N/A
Acute Tox 2 (Fatal if…)	>10%	<35%	>38%
Acute Tox 3 (Toxic if…)	>1.66% to <10%	>7% to <35%	>19% to <38%
Acute Tox 4 (Harmful if…)	>0.25% to <1.66%	>3.5% to <7%	>3.8% to <19%
Not classified	<0.25%	<3.5%	<3.8%

9. CLP also sets harmonised classification and labelling for **salts of nicotine**, (Table 2) which differs from the harmonised classification of nicotine in that it is regarded as more hazardous:

- Acute Tox. 2; H300 – Fatal if swallowed
- Acute Tox. 1; H310 – Fatal in contact with skin
- Acute Tox. 2; H330 – Fatal in inhaled
- Aquatic Chronic 2; H411 - Toxic to aquatic life with long lasting effects

Significantly, no ATEs are set for salts of nicotine which often results in mixtures that contain a salt of nicotine being classified as significantly more hazardous than their nicotine 'counterpart':

Table 2. CLP hazard classifications for mixtures containing nicotine salts (source HSE)

Salts of nicotine	**Exposure route**		
Hazard category	**Oral**	**Dermal**	**Inhalation (dusts/mists)**
Acute Tox 1 (Fatal if…)	N/A	>10%	N/A
Acute Tox 2 (Fatal if…)	>10%	>2.5% to <10%	>10%
Acute Tox 3 (Toxic if…)	>1.66% to <10%	>0.5% to <2.5%	>5% to <10%
Acute Tox 4 (Harmful if…)	>0.25% to <1.66%	>0.25% to <0.5%	>1% to <5%
Not classified	<0.25%	<0.25%	<1%

10. Again, some suppliers may apply classifications that vary from those above with justifiable reasons. In addition, e-liquids containing a salt of nicotine may still contain quantities of (unreacted) nicotine or acid, which must be taken into account when considering the hazardous of the e-liquid. In particular, some acids present significant human health hazards in their own right, e.g. benzoic acid is classified as

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having specific target organ toxicity (STOT) on prolonged or repeated exposure in that it damages the lungs through inhalation.

Estimating the number of nicotine salts in ENDS products on the UK market (provided by MHRA)

11. There are a total of 1409 unique notified e-cigarette products which list a nicotine salt in their ingredients. The most popular nicotine salts reported was Nicotine Salicylate with over 700 products on the published list, followed by Nicotine Lactate and Nicotine Benzoate with around 300 each. Other nicotine salts had much lower numbers of products, if any at all.

12. Anecdotally it has been shown that submitters will record the ingredients of their products either as the nicotine salt, or with the nicotine and acid split in the ingredients. The latter might be to avoid looking like there is more than 20mg of nicotine (as nicotine salt has a higher molecular weight than freebase nicotine), or because this reflects the ingredients which goes into the product, with reactions to form nicotine salts occurring as part of the manufacturing process. This makes establishing the number of salts products on the UK market difficult, the acids used in nicotine salts may be used as ingredients with other functions in the final product. Citric acid is commonly used as a flavouring, however we noted three submissions on the EU Common Entry Gate (EU CEG) portal with nicotine citrate as an ingredient. Citric acid due to its established flavouring properties was not included in any further notification analysis.

13. In all cases there were more products on the EU CEG portal than were on the published notified products list. Submitted products may not be published for a number of reasons including removal from the UK market, decisions not to market products or because they did not pass the internal MHRA notification checks for completeness.

14. For the products which list one of the acids known to be used to form nicotine salts in the ingredients, rough calculations based on the minimum amount[1] for one to one molar reaction ratio were done to estimate the amount of the products containing salt forming acids which could be nicotine salt products. From these calculations of presumed salt products, again salicylic acid containing was the most prevalent (estimated 558 additional products) followed by benzoic acid (405) and lactic acid (402). There is approximately 1600 presumed nicotine salts products in addition to the 1409 reported to contain a nicotine salt. This however is only a ‘best guess’ based on reported ingredients and is unlikely to be entirely accurate.

The impact of pH on nicotine pharmacokinetics; reviews and commentaries

15. Absorption of nicotine across biological membranes is pH dependent. Nicotine is a weak base with pKa 8.0 and in the ionised state (in acidic conditions) does not

[1] Rounded down to 1 decimal place to account for potential rounding differences in the reporting of ingredients.

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rapidly cross membranes. Absorption in the mouth is thus dependent on the pH of the smoke or aerosol inhaled; at alkaline pH, a considerable proportion of nicotine is in the freebase form, which is well absorbed through the mouth. Nicotine that reaches the small airways and alveoli is rapidly absorbed at the lung fluid pH of 7.4, leading to a rapid rise in blood concentrations and delivery to the brain within 10-20 seconds of inhalation. Nicotine absorption from chewing tobacco or snuff occurs more slowly, with blood concentration peaking at around 30 min after use. Nicotine replacement therapies (NRT) (transdermal patches, nasal sprays, inhalers, sublingual tablets, lozenges) are buffered to alkaline pH, nevertheless absorption is slower than from conventional cigarette (CC) smoking, with nasal spray providing the most rapid form of absorption. Nicotine undergoes first-pass metabolism following ingestion, reducing its bioavailability. Nicotine is well absorbed through skin, with a time lag of approximately 1 h between application of a transdermal patch and appearance of nicotine in the blood. Percentage bioavailability for nicotine administered as single doses by various routes has been reported as follows: smoking 1 CC (80-90%); i.v. approximately 5.1 mg (100%); nasal spray 1 mg (60-80%); gum 2-4 mg (55-78%); inhaler 4 mg (51-56%); lozenge 2-4 mg (50-79%); transdermal patch 14-21 mg/24 h (68-100%); s.c. injection 2.4 mg (100%); oral capsule 3-4 mg (44%); oral solution approximately 3 mg (20%); enema approximately 3.5 mg (15-25%) (information in this paragraph is taken from reviews by Hukkanen, Jacob and Benowitz (2005), Benowitz, Hukkanen and Jacob (2009)).

16. In a recent publication, Duell, Pankow and Peyton (2019) discussed the history of the development of traditional combustible tobacco products with relation to pH and the presence of different chemical forms of nicotine (this publication also reported experimental studies on nicotine salt-containing ENDS products, which are described later in this review (paragraph 24). Tobacco that was initially exported to England from Virginia in the early 1600s was in the dark, ‘air-cured’ form, produced by slow drying in ventilated barns. During the drying process, leaf sugars, the precursors of tobacco smoke organic acids, are lost, leading to the formation of a product that produces smoke with a relatively basic pH and high proportion of freebase nicotine. Freebase nicotine is very volatile and associated with a harsh characteristic on inhalation. In the 1850s, the process of ‘flue-curing’ tobacco was developed. Yellow, flue-cured tobacco retains a high level of leaf sugars thus the smoke produced contains organic acids, leading to the presence of nicotine in the protonated salt form, and a much lower presence of freebase nicotine. Flue-cured tobacco smoke has a lower pH and is notably milder on inhalation than the smoke produced from air-cured tobacco. The commentary of Duell and colleagues noted that tobacco industry evaluations have generally found smoke pH to be strongly negatively correlated with leaf sugar levels and leaf sugar/leaf nicotine ratios. This manifests in different user behaviour depending on product type; for example flue-cured tobacco smoke, which is likely to have a pH around 5.5-6.0 is generally inhaled into the lungs, whereas cigar smoke, for which the pH is generally higher, is usually retained by the user in the mouth. The discussion of Duell and colleagues also comments on measurements of the fraction of total nicotine that is in the freebase form (α_{fb}) in CC smoke particulate matter (PM) in recent years; ‘American

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blend' CC are predominantly flue-cured, and analysis of smoke PM from nine brands revealed relatively low α_{fb} (in the range of 0.01-0.10), while atypical brands with higher air-cured tobacco content had higher α_{fb} values (Gauloises Brunes, 0.25; American Spirit/Maroon, 0.36) (Pankow et al. 2003 *cited in* Duell et al 2019).

17. Duell et al. (2019) provided a representative figure summarising the historical changes in tobacco production relating to pH and correlating this with similar developments that have taken place in the development of nicotine salts for use in ENDS. This figure is reproduced in Figure 1, below.

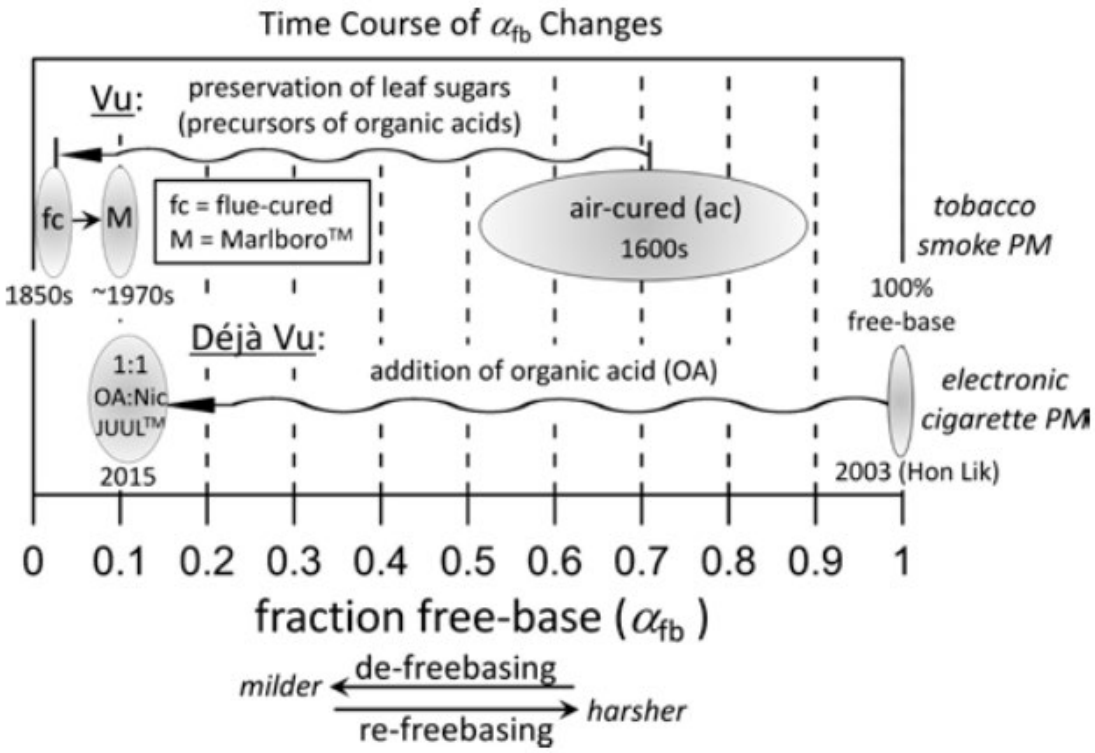

Figure 1. A representation of the historical changes in the proportion of freebase nicotine in tobacco smoke particulate matter (top) and comparison with development of ENDS fluids and aerosols (bottom). fc, flue-cured; αfb, fraction of nicotine in the free-base form; M, Marlboro; Nic, nicotine; OA, organic acid; PM, particulate matter (reproduction of Figure 3 from the publication of Duell et al. (2019)).

18. Keithly et al. (2005) published a review of the use and effects of levulinic acid as a tobacco additive, based on a systematic search of databases of internal tobacco industry documents that have been made available in the public domain following the 1998 Master Settlement Agreement between US state attorneys general and major U.S. tobacco manufacturers. The review notes that the tobacco industry has an established history of manipulating the pH levels of tobacco and smoke. In the early 1970s, ammonia was used to increase smoke pH levels, based on the belief that higher pH would increase the impact of nicotine and the rate at which it is absorbed into the bloodstream (by increasing the proportion of nicotine in the freebase form). Data from internal studies also indicated that companies

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explored the use of organic acids for manipulation of nicotine delivery in CC smoke, noting studies involving lactic, citric, and tartaric acids. In the late 1980s, a major company filed a patent for the use of levulinic acid. This acid is reported to have a sweet taste, and to improve sensory character (smoothness) of smoke and raise delivered nicotine. Internal documents also suggest that levulinic acid may enhance the binding of nicotine to neurons that would ordinarily be unresponsive to nicotine. This combination of effects corresponds to increased levels of plasma nicotine in smokers of CC treated with levulinic acid. The review of Keithly and colleagues summarise in detail information on these different effects of levulinic acid as a CC additive, namely: altered sensory perception of smoke (decreased throat irritation); decreased smoke pH, increased smoke nicotine delivery and absorption, reduced tar/nicotine ratio, enhanced binding of nicotine in brain.

19. A review article focussing on clinical studies of nicotine delivery via the pulmonary route noted that the pulmonary route is the fastest practical way to deliver nicotine to the brain and that a pulmonary NRT would deliver a rapid bolus of nicotine to ‘peak seekers’ and allow ‘trough maintainers’ to titrate their nicotine intake precisely to suit their nicotine metabolism and nicotinic acetylcholine receptor (nAChR) sensitivity (Caldwell, Sumner and Crane 2012). This review summarised studies that had delivered nicotine using nebulisers, unmetered inhalers, ENDS, metered-dose inhalers, and other, novel devices. It was noted that challenges to successful delivery of nicotine via the lung include optimisation of nicotine particle size and mass, and minimisation of aversive effects. With relation to the latter aspect, nicotine inspiration can provoke upper airway stinging, burning, piquancy, prickling, freshness, and tingling, and can cause dose-dependent cough or reflex interruption of inspiration. Limitation of irritation from inhaled nicotine may be achieved by either reducing the amount of nicotine impacting on the oropharynx and upper airway, adding a flavouring (as a diversion), viewing the irritation as desirable, and/or adding a nonaversive envelope coating to the nicotine. One way in which a reduction in the amount of nicotine impacting the back of the throat can be achieved is to reduce the pH to form a nicotine salt, as aerosol at pH 5.5 is more readily inhaled into the lungs than aerosol at neutral pH (Lux & Frecker 1988, *cited in* Caldwell et al. (2012). Another advantage of delivering nicotine at a reduced pH was noted to be the avoidance of alkaline activation of protective mechanisms located in the airway and carotid bodies, thus preventing alkaline damage to the airways. Overall, Caldwell et al concluded that pulmonary nicotine delivery might be maximised by the use of nicotine salts, which have a more physiological pH than pure nicotine, and also by optimising particle mass for alveolar absorption, and adding flavouring agents. This would be aided by the use of metered-dose inhalers.

Analytic studies of nicotine salts in ENDS products

20. Literature searches identified five publications that reported analytical evaluation of ENDS e-liquids and/or aerosols with a specific focus on content of nicotine and organic acids.

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21. El-Hellani et al. (2017) conducted high-performance liquid chromatography (HPLC) analysis of some nicotine-containing e-liquids purchased via the internet. Findings are summarised in Table 3, below.

Table 3. Identification of counter-anions of protonated nicotine in some commercially obtained e-liquid solutions (data from El-Hellani et al. 2017).

Brand	Flavour	Listed nicotine concentration (mg/mL)	pH	Counter anion
EC-blend	Buttered Keoke rum coffee	18	5.5	Acetate
Blu	Classic	24	7.7	Acetate
Retro	Citrus fizz	6	9.0	Acetate
Retro	Juggle bear	18	7.0	Acetate
Liquid express	Watermelon chill	26	9.1	Citrate

22. Based on these findings, the authors conducted studies of standard solutions of nicotine acetate, nicotine citrate, and nicotine formate in PG, prepared in the laboratory to a pH of 8 and nicotine concentration of 36 mg/mL, to assess the effect of the counter-anion on nicotine emissions from e-liquid to aerosol under equivalent operating conditions of an electronic vaporisation device. Although nicotine yield to aerosol increased with device operating power, it did not differ between the aerosols generated from the nicotine acetate, nicotine citrate, or nicotine formate e-liquids. Nevertheless, the relative proportions of freebase and protonated nicotine were influenced by the type of counter-anion present; heating of nicotine acetate and nicotine formate led to similar proportions of freebase/protonated nicotine in aerosol as in the parent e-liquid, while nicotine citrate produced aerosol with a significantly higher fraction of freebase nicotine than in the parent e-liquid. Authors concluded that the counter-anion of protonated nicotine salts does not influence the total nicotine yield to aerosol but does determine the freebase/protonated nicotine distribution. This would affect the nicotine delivery to different absorption sites in the human respiratory tract, and hence impact on systemic delivery of nicotine.

23. Harvanko et al. (2019) conducted spectrometric analyses of 23 e-liquids[2] advertised as containing nicotine salts to determine the presence or absence of 11 different organic acids: glycolic, pyruvic, lactic, levulinic, fumaric, succinic, benzoic, salicylic, malic, tartaric, and citric. Six of these acids were identified in the

[2] n=21 liquids marketed for second or third generation products; n=2 disposable ‘pods’ (R J Reynolds’ Vuse; Juul), all products purchased online. The liquids were in a base of PG and/or glycerol.

samples analysed: lactic (in n=11 liquids), benzoic (n=8), levulinic (n=4), salicylic (n=2), malic (n=2), and tartaric (n=1). Most liquids contained only one type of acid, but three of the liquids contained either two (benzoic + levulinic; benzoic + malic) or five (benzoic + levulinic + salicylic + malic + tartaric) acids. One liquid did not contain any of the organic acids investigated. Across the 23 e-liquids, nicotine concentrations ranged from 40-100 mg/mL (label) or 28.9-88.6 mg/mL (measured), and sample pH ranged from 3.45-6.83.

24. Duell et al. (2019) performed nuclear magnetic resonance (NMR) analyses of a range of 29 commercially available e-liquids to determine the level of organic acid(s) and nicotine in the product and the fraction of nicotine in the freebase form (α_{fb}). Samples examined included various JUUL 'pods', 'look-a-like/knock-off' pods and bottled 'nicotine-salt' and 'non-salt' e-liquids, purchased in the US. Values of α_{fb} for JUUL products, which are based on nicotine + benzoic acid, were generally around 0.1. The authors noted that the 'de-freebasing' (i.e. reduction of α_{fb} from 1.0 to around 0.1) of ENDS products is following a similar history to that of conventional (tobacco) cigarettes (CC), where nicotine was shifted to a lower proportion of freebase over time to improve palatability (see paragraphs 16-17). The authors commented that de-freebasing of ENDS nicotine would be likely to make these products more effective quitting aids for CC smokers, however, it would also be likely to make them more addictive for never-smokers. Following on from this publication, Pankow, Duell and Peyton (2020) reported the development of a framework for predicting α_{fb} values in e-liquids, noting that these values cannot be measured using methods that involve significant dilution of test liquids with water.

25. Talih et al. (2019) analysed various characteristics, including nicotine content and pH, of three tobacco-flavoured JUUL pods purchased in the US. Mean measured values over the three samples were reported as follows: nicotine concentration in e-liquid, 68.6 mg/mL; ratio of protonated/freebase nicotine in aerosol, 94/6; e-liquid pH, 5.4; PG/glycerol ratio, 31/69. This limited report did not provide any further information of relevance.

26. Mallock et al. (2020) conducted laboratory analyses on ENDS 'JUUL pods', including the 'American version' (nicotine content described as 58 mg/mL), 'European initial version' (labelled as containing 20 mg/mL nicotine), and a newer 'European modified version' which the authors noted may be referred to as 'Turbo' (labelled as containing either 9 or 18 mg/mL nicotine), marketed in Germany. The publication of Mallock et al. describes this latter device as functioning with the same power delivery parameters as the European initial version, but with a different wick material, allowing for an increased vaporisation and liquid supply rate. Sample pods of the American version (Virginia Tobacco flavour), European-initial version (n=5 flavour types: Rich Tobacco, Royal Crème, Mint, Mango, Apple), and European modified version (Rich Tobacco flavour) were evaluated for the presence of various analytes in e-liquid and/or in aerosol produced under equivalent puffing regimens. Results are summarised in Table 4, below.

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Table 4. Characterisation of JUUL-pod liquids and aerosols (data from Mallock et al 2020).

	American (Virginia Tobacco flavour[a])	European Initial (range measured across n=5 flavour-types[b])	European Modified (Rich Tobacco flavour)	European Modified (Rich Tobacco flavour)
Labelled nicotine content (mg/mL)	58	20	9	18
Measured nicotine content (mg/mL)	NR	17.20 – 17.78	9.03	17.69
Benzoic acid concentration (mg/mL)	NR	8.82 – 9.64	7.02	12.67
Molar ratio nicotine:benzoic acid	NR	1:0.7	1:1.0	1:1.0
pH (1:20 dilution in ultrapure water)	NR	5.42 – 5.74	5.40	5.42
Total particulate mass (TPM) in aerosol (mg/puff; mean of first 160 puffs)	1.4	1.6 – 1.8	3.7	3.7
Nicotine delivery to aerosol (µg/puff; mean of first 160 puffs)	72	23 - 24	30	61
Benzoic acid delivery to aerosol (µg/puff; mean of first 160 puffs)	NR	21[c]	22	41

[a] Further details for analysis of the American product were not reported. [b] European-modified pods were tested for the following five flavour-types: Rich Tobacco, Royal Crème, Mint, Mango, Apple. [c] Only reported for Rich Tobacco flavour. NR, not reported.

27. Measured nicotine content was in the range of 17-18 mg/mL across the range of five flavours of European-initial pods (all labelled as 20 mg/mL) and the 18 mg/mL-labelled European-modified pod[3]. Benzoic acid content was higher (12.67 mg/mL) in the 18 mg/mL-labelled European modified pod than in the range of

[3] Except for one sample-type of European-modified pod for which the listed and measured nicotine concentration was 9 mg/mL.

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five flavours of 20 mg/mL-labelled European initial pods (approximately 9 mg/mL). Thus, the nicotine/benzoic acid ratio was lower in the European-modified pods compared with European-initial pods. Mean nicotine delivery to aerosol from American pods was 72 ± 25 µg per puff, which was approximately three-fold the level measured from European-initial pods. European-modified pods generated more than double the amount of aerosol (total particulate matter, TPM) per puff as either European-initial or American pods, with the result that nicotine delivery to aerosol from European-modified pods was in an equivalent range to that from American pods, despite the much lower nicotine concentration in the e-liquid of the European-modified pods.

28. The authors of this study commented that their data supported an assertion that it may be more useful for regulatory authorities to set limits based on amount of nicotine delivered to aerosol, rather than for concentration of nicotine in e-liquid. This would, in particular, provide some level of protection against nicotine addiction for novice ENDS users who initiate use of pod-based products (e.g. adolescents), although the authors also acknowledged that higher delivery of nicotine to aerosol may be useful to people who use ENDS as an aid to quitting CC smoking.

Pharmacokinetics of products containing nicotine salts

29. Literature searches also identified a small number of reports describing studies of the pharmacokinetics of nicotine in salt form inhaled from aerosol-generating products, including four small-scale clinical studies in CC smokers, and one study in rats.

Studies in human CC smokers

30. Rose et al. (2010)[4] conducted a double-blind, placebo-controlled crossover study that compared plasma nicotine levels achieved after use of a prototype nicotine pyruvate aerosol generation system (NP) or a Nicotrol/Nicorette nicotine vapour inhaler cartridge (NV) in nine regular CC smokers (≥10 CC/day). The NP system comprised an aerosol-generation apparatus that produced aerosol with a median mass aerodynamic diameter (MMAD) of approximately 600 nm from a mixture of pyruvic acid and free base nicotine, without inclusion of other chemicals (e.g. PG).

31. Following overnight abstinence from nicotine-containing products, five test conditions were evaluated during a 5.5 h period on the study day, at 50 min intervals, as follows: delivery of 10, 20, or 30 µg nicotine, as nicotine pyruvate, per 35 mL puff from a measured dose delivery system (NP10, NP20, NP30); delivery of room air via the same measured-dose delivery system; delivery of 10 µg nicotine from a commercially obtained Nicotrol/Nicorette inhaler (NV). In each case, a total of 10 puffs were taken at 30 s intervals. NP doses were presented in ascending order (NP10, NP20, NP30) and were alternated with control conditions in a counter-balanced sequence. Venous plasma nicotine levels were determined before each

[4] This study was supported by Philip Morris U.S.A.

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test (baseline) and during a 30 min period starting 5 min after the final puff. The highest nicotine levels were observed at the 5 min time point, thus subsequent evaluation of results compared the change from baseline to 5 min post inhalation.

32. Baseline nicotine levels ranged from 1.0-2.7 ng/mL. NP20 and NP30 conditions produced significant increases in 5 min plasma nicotine, while peak nicotine concentrations were not detected for placebo, NV, or NP10 conditions. Mean increases in plasma nicotine levels at 5 min post inhalation for NP20 and NP30, respectively, were 5.0 and 8.3 ng/mL vs. baseline, 4.8 and 8.0 ng/mL vs. placebo at 5 min post inhalation, and 4.7 and 6.6 ng/mL vs. NV at 5 min post inhalation. The authors noted that one CC would typically be expected to supply a dose of approximately 1 mg nicotine and produce a venous plasma nicotine peak of 10-15 ng/mL. Participants rated the NP test products as more satisfying and providing greater withdrawal relief compared with placebo but not compared with NV. There were no significant adverse reactions. Comparison of NP20 and NP30 with NV indicated a significantly lower level of harshness/irritation for NP20 compared with NV. Authors concluded that the NP aerosol-delivery system produced user satisfaction, was well tolerated, could produce a rapid increase in plasma nicotine concentration and deliver a higher dose than a vapour-delivery system (nicotine inhaler).

33. Teichert et al. (2018)[5] evaluated pharmacokinetics and subjective effects following use of the nicotine lactate aerosol delivery system ('P3L') in comparison with a Nicorette inhaler. This work was described as a follow-on of development from the 'NP' product tested by Rose et al. (2010) (paragraphs 30-32). Lactic acid was chosen to replace pyruvic acid due to the greater stability of lactic acid during heating in addition to the desirable safety profile of lactic acid and the ability to generate an aerosol upon mixing with nicotine vapour. The study aims were to evaluate, in healthy CC smokers, the plasma nicotine pharmacokinetic profile, subjective effects, safety and tolerability of nicotine-containing aerosol delivered by P3L in comparison with the Nicorette inhaler.

34. Tests were conducted on separate days and study participants (n=14 smokers of ≥10 CC/day) abstained overnight prior to each study day. On day 1, participants took 80 puffs of a Nicorette inhaler over 20 min (1 puff/15 s). Subsequent visits tested three variants of P3L delivering 50, 80, and 150 µg nicotine/puff, with a total of 12 puffs taken over 6 min (1 puff/30 s). There was a minimum interval of 12 h between visits and doses were tested in ascending order. Puff duration and volume were not controlled. Nicotine venous plasma concentrations were determined over a period from 45 min before to 240 min after the start of test product use, and maximum baseline-corrected plasma concentration (C_{max}), time to C_{max} (t_{max}), and baseline-corrected area under the plasma concentration-time curves from start of product use (t_0) to last quantifiable nicotine concentration time point ($AUC_{0\text{-last}}$) and from t_0 to 10 min after t_0 ($AUC_{0\text{-}10}$) were

[5] This study was supported by Philip Morris Products S.A.

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determined. Pharmacokinetic parameters were analysed for the three P3L variants and the Nicorette inhaler using analysis of variance (ANOVA) with product exposure as a fixed effect and subjects as a random effect, adjusted for sex.

35. Plasma nicotine concentration-time curves are shown in Figure 2, and data in Table 5. Curves obtained after P3L use were similar for all three nicotine levels, with C_{max} of 9.7, 11.2, and 9.8 ng/mL for the 50, 80, and 150 µg/puff products, respectively, at a t_{max} of 7.0 min. For the Nicorette inhaler, C_{max} of 6.1 ng/mL was reached at t_{max} of 30 min. $AUC_{0\text{-last}}$ was 9.9, 10.3, and 10.0 h x ng/mL for 50, 80, and 150 µg/puff P3L, respectively, and 12.3 h x ng/mL for Nicorette inhaler. However, $AUC_{0\text{-}10}$ values were higher for P3L (1.0, 1.2, and 1.0 h x ng/mL for 50, 80, and 150 µg/puff, respectively) compared with Nicorette inhalator (0.1 h x ng/mL). Authors noted that C_{max} and t_{max} values produced by P3L were in a range comparable to published data for CC. P3L was well tolerated and was associated with a slightly higher (faster) craving reduction compared with Nicorette. No serious adverse events were reported.

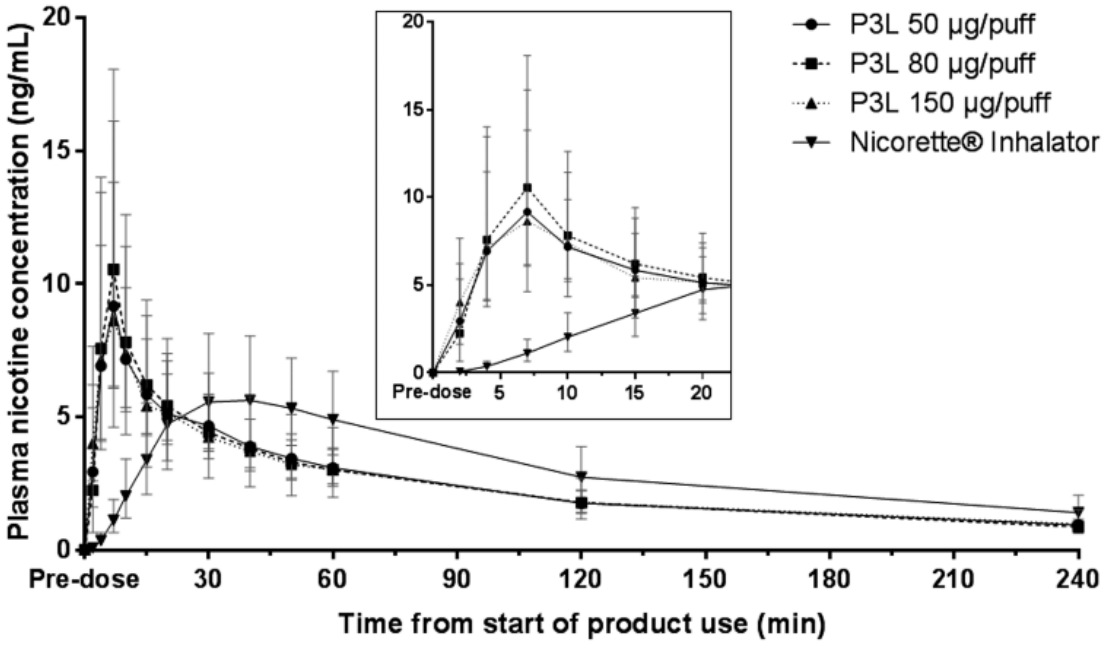

Figure 2. Geometric means and 95% confidence intervals of baseline-corrected nicotine concentrations during single use of the P3L system (50, 80, 150 µg/puff) and Nicorette inhaler over 4 h, and expanded view from t_0 to 20 min (reproduction of Figure 1 from Teichert et al (2018)).

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Table 5. Pharmacokinetics of nicotine following single use of the P3L system of the Nicorette inhaler (reproduction of Table 1 from Teichert et al. 2018)

Parameter	P3L (50 µg/puff), *n* = 15	P3L (80 µg/puff), *n* = 14	P3L (150 µg/puff), *n* = 14	Nicorette inhalator (15mg), *n* = 15
C_{max}, ng/mL				
Geometric LS mean (95% CI)[a]	9.7 (6.7, 13.9)	11.1 (7.7, 16.1)	9.8 (6.8, 14.2)	6.1 (4.2, 8.8)
Min, Max	1.7, 18.7	1.4, 20.8	0.8, 33.3	1.7, 17.7
t_{max}, min				
Median	7.0	7.0	7.0	30.0
Min, Max	4.0, 30.0	4.0, 20.0	2.0, 20.0	20.0, 60.0
$AUC_{0-10'}$, h×ng/mL				
Geometric LS mean (95% CI)[a]	1.0 (0.6 to 1.7)	1.2 (0.7 to 1.9)	1.0 (0.6 to 1.7)	0.1 (0.1 to 0.2)
Min, Max	0.1, 2.3	0.1, 2.4	0.04, 4.1	0.02, 0.4
AUC_{0-last}, h×ng/mL				
Geometric LS mean (95% CI)[a]	9.9 (7.5 to 13.2)	10.3 (7.6 to 13.8)	10.0 (7.4 to 13.4)	12.3 (9.3 to 16.4)
Min, Max	3.4, 19.7	2.8, 17.7	1.6, 31.7	3.3, 33.7

C_{max} = baseline-corrected plasma concentration; t_{max} = time to reach C_{max}; AUC = baseline-corrected area under the plasma concentration–time curves
[a]Geometric means and 95% confidence interval (CI) are the adjusted geometric least squares means and CIs from an ANOVA model conducted on log-transformed data with product as fixed effect and subjects as random effect, adjusted for sex. Values are derived from baseline-corrected nicotine concentrations.

36. O'Connell et al. (2019)[6] reported a study which aimed to evaluate and compare parameters of nicotine kinetics and user experience across the use of ENDS products containing nicotine in either the freebase form or as the lactate salt. The study was a randomised, open-label, six-period crossover study including 15 regular CC smokers. Products tested were described as follows: (1) participants own brand of CC; (2) *my*blu pod-system containing 25 mg nicotine ('freebase') tobacco flavour; (3) *my*blu pod-system containing 16 mg nicotine lactate tobacco flavour; (4) *my*blu pod-system containing 25 mg nicotine lactate tobacco flavour; (5) *my*blu pod-system containing 40 mg nicotine lactate tobacco flavour; and (6) blu PRO open system containing 48 mg nicotine lactate tobacco flavour. Although the report specifies product parameters for nicotine in units of mg, it is likely that this is a reporting error and it is assumed that the values described actually refer to the nicotine concentration (mg/mL) in the e-liquid.[7]

37. Tests were conducted over six consecutive days, with overnight abstinence from CC smoking or other forms of nicotine exposure prior to each test day. On day 1, all participants smoked one own-brand CC, with puffs taken every 30 s (9 or 10 puffs total). On each of days 2-6, participants used one of the five different ENDS products, in a randomised order, in a protocol described by the authors as '10 inhalations every 30 s for 3 s in duration'[8]. In a small number of cases, participants took only took 9 puffs in total (myblu 25 mg; blu PRO 48 mg). Plasma nicotine was monitored before, during, and after test sessions, and reported as maximum concentration (C_{max}), median time to C_{max} (T_{max}), and mean area under the curve

[6] This study was conducted by Imperial Brands plc / Fontem Ventures B.V., manufacturers of the 'blu' series of E(N)NDS products.
[7] The 'Methods' section notes that *my*blu pods contain 1.5 mL e-liquid and delivers an average of 7-8 mg aerosol per puff, while blu PRO has a refillable clearomiser with 2.0 mL capacity and delivers an average of 2-3 mg aerosol per puff.
[8] The wording of puffing protocol given in the 'Procedure' section of the publication is unclear. However, it is assumed to be most likely that the protocol was for participants to take one 3-s inhalation every 30 s, for a total of 10 inhalations.

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from time 0-30 min (AUC_{0-30}). Participant subjective effects, such as desire to smoke, nicotine satisfaction, and dizziness, were also recorded on a Likert-type scale.

38. Nicotine pharmacokinetic profiles reported by O'Connell et al., are shown in Table 6 and Figure 3, below[9].

Table 6. Pharmacokinetic parameters measured by product type (reproduction of Table 3 from O'Connell et al (2020)).

	Conventional cigarette	*my*blu 40 mg (nicotine lactate)	*my*blu 25 mg (nicotine lactate)	*my*blu 16 mg (nicotine lactate)	blu PRO 48 mg (nicotine lactate)	*my*blu 25 mg (freebase)
C_{max}, ng/mL	17.81 (49.6)	10.27 (83.6)	7.58 (80.6)**	6.51 (76.5)***	4.85 (108.3)***, †	5.048 (49.9)***
T_{max}, median (range), min	8.05 (5.00–15.13)	7.9 (1.97–15.0)	6.03 (4.58–16.77)	6.967 (3.98–15.05)	6.908 (2.35–15.03)	8.034 (2.28–15.10)
AUC_{0-30}, ng*min/mL	324.9 (35.8)	190.7 (71.8)	125.2 (53.4)***	118.5 (60.8)***	84.84 (89.8)***, ††	98.99 (35.8)***

All values are geometric mean and geometric coefficient of variation (CV %) unless stated otherwise

C_{max} maximum plasma nicotine concentration; T_{max} time to maximum nicotine concentration; AUC_{0-30} area under the concentration–time curve from time zero to the last quantifiable concentration (30 min)

*Significant difference compared to conventional cigarette (**$P<0.01$ and ***$P<0.001$)

†Significant difference between *my*blu 40 mg (nicotine lactate) and blu PRO 48 mg (nicotine lactate) (†$P<0.05$; ††$P<0.01$)

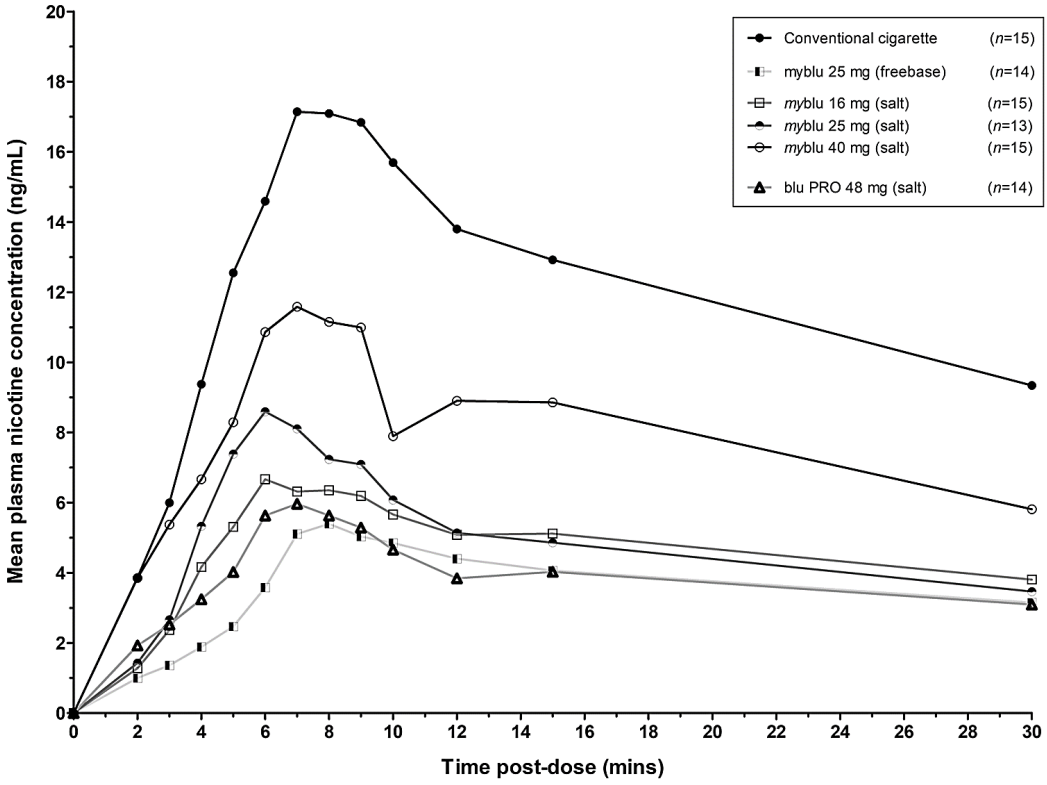

Figure 3. Pharmacokinetic profiles: mean plasma nicotine concentration by investigational product (linear scale) over 30 min. (reproduction of Fig. 1 from O'Connell et al (2020)).

[9] http://creativecommons.org/licenses/by/4.0/

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39. Nicotine pharmacokinetics achieved from CC smoking were most closely represented under use of the 40 mg nicotine lactate ENDS product. For subjective effects, relief of desire to smoke and experiencing dizziness were associated with rapid nicotine absorption and higher C_{max}; other effects were not significantly different across the test products. Some adverse events were reported: one case of insomnia after use of 48 mg blu PRO (nicotine lactate); one case of headache after use of 25 mg *my*blu (freebase); a further nine adverse events that were considered to be unrelated to study product use.

40. Authors suggested that the 40 mg nicotine lactate formulation could be an effective CC-quitting aid and proposed that the upper limit of 20 mg/mL nicotine stipulated by the European Union Tobacco Products Directive (EUTPD) should be reviewed.

41. A randomised, open-label, parallel-cohort study reported by Jay et al. (2020) investigated the effect of switching to a 'JUUL' nicotine-salt pod system on various biomarkers of exposure related to CC smoking. Adult CC smokers (≥10 CC/day) who had never used ENDS products were randomised to one of six groups (n=15/group), as follows: *ad libitum* use of one of four flavours of JUUL pod (Virginia Tobacco, Mint, Mango, Crème); *ad libitum* continuation of usual-brand CC smoking; or abstinence from nicotine products. The JUUL nicotine-salt pod system was described as a closed ENDS product that delivers aerosol to the user through the vaporisation of an e-liquid containing 0.7 mL of a 5% nicotine salt solution containing PG, glycerol, flavourants, nicotine (59 mg/mL; 40 mg/pod) and benzoic acid. The study was conducted for a total of nine days under clinical confinement, with a treatment test period of five days. Total nicotine equivalents and nine other CC smoking-related biomarkers of exposure (BoE)[10] were measured in 24-h urine collections at baseline (i.e. when all participants were still smoking their usual-brand CC) and after five days of test treatment. Nicotine dependence, urge to smoke, and adverse events were also recorded.

42. Subjects in the usual-brand CC group consumed an average of 19.3 CC/day, while average e-liquid consumption was 0.79 g/day. From baseline to study end, mean total urinary nicotine equivalents (mg/24 h) decreased by 96.4% in the abstinence group. A significant increase was noted for JUUL-Mango (+25.3%, p=0.045), while non-significant changes were noted for JUUL-Crème (+14.9%), JUUL-Mint (+2.9%), JUUL-Virginia Tobacco (-6.5%) and usual-brand CC (+26.1%). On day 5, means total urinary nicotine equivalents were 18.3 mg/24 h for pooled JUUL cohorts and 19.0 mg/24 h for usual-brand CC cohort (baseline values were not reported).

43. Significant reductions in non-nicotine BoEs over the study period were noted in groups switching to JUUL or to abstinence (approximately 85% reduction over an aggregate of 8 BoEs for the pooled JUUL cohort and for the abstinence cohort), while BoEs increased slightly in the usual-brand CC group. Urge to smoke was

[10] NNN, NNAL, 3-HPMA, MHBMA, S-PMA, HMPMA, CEMA, 1-OHP, COHb

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lowest in the usual-brand CC cohort and greatest in the abstinence cohort, however there was not significant difference between usual-brand CC and JUUL groups on day 5. No serious adverse events were reported.

Studies in animals

44. Phillips et al. (2015) reported a 28-day (OECD 412) inhalation toxicity study of nebulised nicotine, alone or in combination with pyruvic acid, to evaluate local and systemic effects in Sprague Dawley rats. Details of toxicological and transcriptomic analyses conducted in this study were summarised in a previous COT discussion paper (TOX/2019/38) and the description in this current narrative is limited to data of relevance to the comparative toxicokinetics of nicotine, pyruvate, and nicotine pyruvate.

45. Rats (n=10/sex/group) were exposed for 6 h/day, 5 days/week, in a nose-only chamber, to either filtered air (Sham) or aerosols of phosphate-buffered saline vehicle (PBS) at physiologic pH; 50 µg/L [50 mg/m^3] nicotine (Nic); 33.9 µg/L pyruvate (Pyr); 18 µg/L nicotine/9.8 µg/L pyruvate, 25 µg/L nicotine/13.6 µg/L pyruvate, or 50 µg/L nicotine/27.1 µg/L pyruvate (Nic/Pyr groups). Aerosol MMADs were in the range of 1.4–2.0 µm (GSD 1.7–2.2). The authors calculated that a nicotine concentration of 50 µg/L represented a delivered dose (DD) to the rat of 13.6 mg/kg bw[11], and a human equivalent dose (HED) of 2.2 mg/kg bw[12]. This was considered to be equivalent to a daily dose of nicotine equivalent to smoking approximately 130 CC (132 mg for a 60 kg person).

46. Rats exposed to 50 µg/L Nic or 50/27.1 µg/L Nic/Pyr showed similar recovery of total nicotine metabolites, which the authors concluded was an indication that the presence of pyruvic acid did not affect nicotine uptake. Slight differences in the distribution of nicotine metabolites were observed for the three Nic/Pyr test atmosphere concentrations but the authors noted that these differences were consistent with the relative variability of metabolites observed in historic studies with CC smoke. Measurement of urinary pyruvate was noted by the authors not to be a reliable method for monitoring uptake of pyruvic acid or sodium pyruvate, in part because it is not possible to distinguish between exogenous and physiologic pyruvate.

Summary/conclusions

47. The nicotine present in ENDS products has predominantly been in the 'freebase' form, however some more-recent products contain organic acids in the e-liquid, leading to the presence of a proportion of the nicotine in the protonated form, as a salt. Nicotine salts are less volatile than freebase nicotine and are reported to produce a less harsh experience on inhalation. Narrative relating to the history of

[11] Calculated by the authors as follows: DD = (C X RMV X D) / BW, where DD = delivered dose (mg/kg); C = concentration of substance in air (mg/L); RMV = respiratory minute volume (L/min); D = duration of exposure (min); and BW = body weight (kg), [DD = (0.05 mg/L X 0.194 L/min X 360 min) / (0.25 kg) = 13.6 mg/(kg BW), or 3.4 mg/rat (250 g BW)].
[12] Calculated as follows: HED = DD / 6.6 (from CDER 2005).

development of combustible tobacco products suggests that tobacco production procedures such as 'flue-curing' that allow higher levels of retention of leaf sugars, the precursors of organic acids in tobacco smoke, have led to products that are less harsh to smoke and thus more likely to be inhaled into the lungs rather than kept in the mouth. Information from internal tobacco industry documents that have been made available to the public also indicates that during the second half of the twentieth century, organic acids were tested/used as tobacco additives to reduce pH and enhance smoothness of CC smoke. Thus, it could be expected that the use of ENDS products containing nicotine salts under similar puffing parameters to those containing only freebase nicotine might similarly lead to a higher delivery of nicotine to the lungs rather than the mouth and buccal cavity.

48. Literature searches identified a small number of studies that had analysed the presence of nicotine salts in ENDS products and a few small-scale clinical studies that had investigated the pharmacokinetics of nicotine on inhalation from these types of products.

49. Analytical studies indicated the presence of various organic acids in commercially available e-liquids, including lactic, benzoic, levulinic, salicylic, malic, and tartaric acids. Available data indicated that the majority of nicotine in these products was in the protonated form, with only a small fraction of freebase nicotine.

50. Clinical studies, conducted by product developers, have evaluated the pharmacokinetics of inhaled aerosolised nicotine salt-containing products in comparison with inhalation of products providing nicotine in the freebase form, in small cohorts of regular CC smokers. These generally indicated higher and/or faster nicotine delivery to the user from products containing nicotine and organic acids than from products containing equivalent concentrations of nicotine in the freebase form. However, the identified evidence base was small.

Questions for the Committee

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

i. From the limited evidence base identified, can any conclusions be drawn regarding possible differences in nicotine exposure levels or patterns for users of ENDS products that contain nicotine in salt form as compared with products containing freebase nicotine?

ii. Does this evidence base indicate any additional risks from use of nicotine salts rather than freebase nicotine in e-liquids?

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat November 2020

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Abbreviations

CC	Conventional cigarette
DD	Delivered dose
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
EUTPD	European Union Tobacco Products Directive
GSD	Geometric standard deviation
HED	Human equivalent dose
MMAD	Median mass aerodynamic diameter
nAChR	Nicotine acetylcholine receptor
NRT	Nicotine replacement therapy
PG	Propylene glycol
PM	Particulate matter
TPM	Total particulate mass

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

A summary of data published to date on the presence and pharmacokinetics of nicotine salts in electronic nicotine delivery systems (ENDS) products.

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

Searches were carried out on 05/06/2020 to identify literature 'nicotine salts' and 'e-cigarettes' or 'electronic nicotine delivery systems (ENDS)', as follows:

Scopus

(TITLE-ABS-KEY ("nicotine salt*") AND TITLE-ABS-KEY ("e-cig*" OR "electronic cigarette*" OR "electronic nicotine delivery system*" OR "e-liquid*")): 8 citations.

PubMed

"nicotine salt*"[Title/Abstract] AND ((("e cig*"[Title/Abstract] OR "electronic cigarette*"[Title/Abstract]) OR "electronic nicotine delivery system*"[Title/Abstract]) OR "e liquid*"[Title/Abstract]): 9 citations.

An updated search of PubMed conducted on 24/08/2020 using combinations of the search terms "nicotine salt" AND "e-cigarette" "electronic cigarette" "electronic nicotine delivery system" identified 111 citations, none of which added further information to publications identified by previous searches.

Further searches were conducted on 09/10/2020, as follows:

PubMed

(Nicotine[Title/Abstract] AND pH[Title/Abstract] AND tobacco[Title/Abstract]): 147

(Nicotine[Title/Abstract] AND pH[Title/Abstract] AND tobacco[Title/Abstract] AND bioavailability[Title/Abstract]): 6

(Nicotine[Title/Abstract] AND pH[Title/Abstract] AND tobacco[Title/Abstract] AND inhal*[Title/Abstract]): 8

SciFinder

(Nicotine AND pH AND tobacco): 419

(Nicotine AND pH AND tobacco AND bioavailability): 10

(Nicotine AND pH AND tobacco AND inhal*): 29

Searches of 'grey literature' were also performed.