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

Statement on the potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes)

Background and scope of the review

1. On request from the Department of Health and Social Care (DHSC) and Public Health England (PHE), the COT has reviewed the potential toxicological risks from electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (collectively abbreviated to E(N)NDS). In the UK, E(N)NDS are advocated as a smoking cessation tool, within the context of reduced risk products for tobacco harm reduction, and the main perspective of the COT review was to evaluate the toxicological risk from intended use, in particular as a nicotine substitute to aid smoking cessation. A general review of the topic was conducted, addressing both the absolute risk associated with E(N)NDS use and the relative risk as compared with smoking conventional cigarettes (CC). The evaluation included both ENDS and ENNDS, considering that users may switch from CC smoking to the use of either of these products in the immediate and/or longer term, as well as the possible risk to bystanders when these products are used.

Review methodology

2. The Committee reviewed a number of topics of relevance to assessing adverse health effects to humans associated with use of E(N)NDS products, including: the constituents that may be present in E(N)NDS products and the aerosols emitted from them; toxicological and epidemiological studies on the principal or commonly identified contents and constituents and assessments of potential risk to users and bystanders associated with exposure to E(N)NDS emissions.

3. The format of discussion papers included systematic reviews, summary overviews of published literature reviews, short data summaries, and follow-on papers focussing in more depth on specific aspects raised during discussions. The evidence base was drawn from literature available in public databases. Due to the very large volume of literature available, publications describing studies conducted using *in vitro* systems were reviewed in less detail for the COT assessment. A list of all discussion papers considered by the COT during the review is given in Annex A. The main aspects of the data presented in these papers and the conclusions drawn by the Committee are summarised in subsequent sections of this statement. The reader is referred to the links to individual discussion papers throughout the text for additional background information.

4. New articles relating to E(N)NDS are being published at a high rate. A brief updated literature search using terms relating to 'e-cigarettes' or 'electronic nicotine delivery systems' conducted towards the end of the COT review identified around 900 new publications for the period mid-2018 to mid-2019, of which more than 130 were directly relevant to areas covered in the COT review (see TOX/2019/50). The Committee considers that the evidence-base used in this review is broadly up to date until mid-2019, with searches undertaken as specified within the individual discussion papers (see Annex A).

E(N)NDS products and use

5. E(N)NDS are battery-powered devices containing a liquid with (ENDS) or without (ENNDS) nicotine ('e-liquid'). The e-liquid is heated on use to produce an aerosol that is inhaled by the user ('puffing', 'vaping'). E(N)NDS were introduced commercially during the 2000s as nicotine-delivery devices. E(N)NDS devices generally comprise a mouthpiece, a cartridge or tank containing e-liquid, a heating element (atomizer), and a battery. Commercially available devices are often categorised by 'generation'. First-generation devices ('cigalikes') resemble conventional cigarettes (CC). These are 'closed' systems that are either disposable or have a replaceable cartridge or 'cartomizer' (combined cartridge and atomizer), but are not refillable. Second-generation E(N)NDS are usually open systems that have less resemblance to tobacco cigarettes, often resembling pens or laser pointers ('vape pens'). They have a high-capacity rechargeable lithium-ion battery and a refillable atomizer (sometimes referred to as a 'clearomizer'). Third-generation models ('advanced personal vapers', 'mods') are also refillable, have very high capacity lithium ion batteries and are highly customisable (different coil options, power settings, tank sizes). Beyond this, fourth and fifth generation devices are now described. This statement is a general review of potential toxicological risks of constituents and emission from these devices, and does not focus on any specific product or generation of product, but is considered to be broadly applicable across all devices. The COT assessment of the toxicological risks from E(N)NDS is on those products produced to good manufacturing standards. Additional risks may pertain to products not meeting these standards.

6. E(N)NDS products were initially developed and marketed as nicotine-delivery devices (ENDS), with the aim that they could be used as an alternative to CC smoking that would more closely mirror the user experience and kinetics of nicotine delivery than other available forms of nicotine replacement therapy (NRT). However, some products do not contain nicotine (ENNDS), and these can also play a role in smoking cessation, given the multi-faceted nature of CC dependence. In the UK, ENDS and ENNDS currently fall under different regulatory systems (see paragraphs 16-21). The extent to which E(N)NDS may be effective as an aid to CC smoking cessation has not yet been established. The most recent Cochrane review, 'Electronic cigarettes for smoking cessation' identified three RCTs that had addressed this aspect. A meta-analysis using data from two of these trials noted a slightly higher likelihood of abstinence from CC smoking at six months in subjects

using ENDS (9%) compared with ENNDS (4%) (relative risk (RR) = 2.29, 95% CI 1.05-4.96 for ENDS compared with ENNDS), although confidence in the result was rated as 'low' (GRADE) (Hartmann-Boyce et al. 2016). The authors concluded that more data were needed.

7. In addition to the variability in available types of E(N)NDS products and the settings under which they can be used (e.g. power output), there is a wide variation in normal usage characteristics between individual E(N)NDS users, including factors such as frequency and pattern of daily use, typical daily puff consumption, nicotine concentrations used in e-liquids, and specific puffing parameters (for example, duration and volume of an individual puff). Typical daily puff consumption from E(N)NDS reported in the literature is variable. For the purposes of this COT evaluation, data were taken from a study of 'vaping behaviour' conducted by Dawkins et al. (2018) in the South-East of England. In this study, mean usage levels ranged from 272 to 338 puffs/day when 20 users used a tank-style ENDS device for one week, at fixed or variable power, with e-liquids containing either 6 or 18 mg/mL nicotine.

8. The scope of the COT review concerned only typical use of E(N)NDS. Risks associated with scenarios where these products are used in a non-standard manner were not addressed; such scenarios could include addition of liquids not intended to be used with these devices, use of drugs or novel psychoactive substances, or operation or use of a device in a manner not intended by the producer.

9. The pace of innovation and development in E(N)NDS is very high, with new types of products continually being brought to market. In line with the lag in the available published literature for the COT review (paragraph 4), there will be a lag between the products for which data from research studies are available and the principal products that are currently marketed. This may also have some impact on the applicability of the findings of research studies, and reviews such as this one, to the current E(N)NDS market. Hence, it is important that the findings of this review are interpreted accordingly.

Contents and other constituents of E(N)NDS liquids and aerosols

10. Data from studies that evaluated the chemical constituents of E(N)NDS liquids and/or aerosols were summarised in TOX/2018/16.

11. The principal contents of most e-liquids are the solvents, propylene glycol (PG) and glycerol, which can be present in ratios ranging from 0:100 to 100:0. Other common constituents are water, nicotine, and flavourings, as well as sweeteners and flavour enhancers. Nicotine concentrations in commercial E(N)NDS products vary. The maximum permitted concentration of nicotine in e-liquids in the UK is 20 mg/mL (see paragraph 16), but products containing higher levels of nicotine are permitted for sale in some other countries. As well as standard contents, some studies have reported the presence of other constituents in e-liquids, including contaminants and impurities, which may have been derived from the e-liquid formulants or the

E(N)NDS device, or other substances that have been added into the e-liquid. In addition, some reports suggest that e-liquid contents are not always true-to-label, e.g. some nicotine concentrations have been reported as divergent from label concentrations, including nicotine being found in liquids reported to not contain it.

12. E(N)NDS aerosol is produced by heating the e-liquid within the E(N)NDS device. Typical temperatures attained during the heating process are reported in the range of 40 and 180 °C. The aerosol comprises two main parts – a particulate (droplet) phase and a gas (vapour) phase. The particulate phase (particulate matter, PM) contains droplets that are formed when components within the e-liquid are heated and vaporise, then condense back into liquid aerosol as the gas cools.

13. Analytical studies have been performed to measure the presence and levels of different substances emitted into E(N)NDS aerosols on puffing (see TOX/2019/39). In these studies, aerosols produced by machine puffing were collected and analysed using a variety of different methods. Measurements have been reported mostly as mass per puff(s), but sometimes as the concentration of the substance in the aerosol. There is a wide variation between studies regarding e-liquid composition and type of E(N)NDS device used, protocols used for the production, collection and analysis of aerosols, and methods of data analysis and reporting. Consequently, it is difficult to compare or integrate results across studies. For the purpose of risk assessments conducted in this COT review, studies reporting the highest average level of the analyte of interest, produced under conditions considered to represent 'standard' product use, were selected. The risk assessments thus use a representative normal exposure scenario with the upper end of the range of average concentrations, but cannot represent the full breadth of the wide spectrum of possible exposures that may occur, particularly given the rapid development of the E(N)NDS market.

14. A number of studies also evaluated levels of emissions into ambient air on E(N)NDS use ('bystander exposure', see TOX/2019/11). These studies also used varying methodologies, test products, and methods of data analysis and reporting. For the purpose of the COT review, for assessment of risk to bystanders, exposure data were taken from the study reporting the highest average ambient air concentration of the analyte of interest reported under experimental conditions of 'typical' product use by human users. Therefore, the same caveats apply to risk assessments of exposure to bystanders as for E(N)NDS users.

15. Exposure to E(N)NDS emissions has also been evaluated in biomonitoring studies. Most of these studies have been performed in E(N)NDS users (see TOX/2019/39) although a few studies have looked at biomarkers of nicotine exposure in bystanders (see TOX/2019/11).

Regulations and guidance

16. In the UK, nicotine-containing electronic cigarettes (ENDS) are regulated under the Tobacco and Related Products Regulations 2016[1] (Part 6 Electronic cigarettes), which is an implementation of the 2014 EU Tobacco Products Directive[2] (TPD) (2014/40/EU) (Article 20). Requirements include: maximum limits for e-liquid nicotine strength (20 mg/mL) and tank or refill capacity (2 mL or 10 mL, respectively); a ban on certain ingredients including colourings, caffeine and taurine; and consistent levels of nicotine delivery under normal use. Other aspects covered by the TPD are packaging and product safety, labelling and packaging requirements, notification and vigilance, advertising, and annual reporting requirements. Further details can be found in TOX/2020/06.

17. Suspected adverse reactions and safety concerns can be reported by consumers to the Medicines and Healthcare products Regulatory Agency (MHRA) via its Yellow Card Scheme[3]. From 20th May 2016 to 9th January 2020, a total of 245 reported effects had been listed, for example cardiac, gastrointestinal, immune, and respiratory effects, general disorders, and injuries. The 245 reported effects came from 84 reports, with generally only one to two reports per effect. Further information is provided in TOX/2020/07.

18. The EU TPD does not cover rules on smoke-free environments, domestic advertising or sales arrangements, age limits for sale, nicotine-free products (ENNDS), or flavourings, which Member States are free to regulate. In England, Northern Ireland, Scotland, and Wales, tobacco products (including nicotine-containing ENDS) are prohibited for sale to, or purchase on behalf of, persons under 18 years. There is currently no legislation restricting the use of E(N)NDS in public places, but guidance is available[4] and voluntary restrictions are in place, with many businesses treating them like CC.

19. Products that do not contain nicotine, which includes ENNDS, are outside the scope of the EU Tobacco Products Directive and thus the UK Tobacco regulations, and do not have to meet their requirements. They will continue to be regulated under the General Product Safety Regulations 2005[5]. This includes liquids to which nicotine may be added by the user.

20. UK guidance relating to ‘e-cigarettes’ for people who wish to stop smoking, as listed on the National Health Service (NHS) website, includes the following statements: “Getting expert help from your local stop smoking service gives you the best chance of quitting smoking for good.” and “Many thousands of people in the UK have already stopped smoking with the help of an e-cigarette. There's growing

[1] http://www.legislation.gov.uk/uksi/2016/507/contents
[2] https://ec.europa.eu/health//sites/health/files/tobacco/docs/dir_201440_en.pdf
[3] https://yellowcard.mhra.gov.uk/yellowcards/tobaccoreportmediator/
[4] https://www.gov.uk/government/collections/e-cigarettes-and-vaping-policy-regulation-and-guidance#advice-for-organisations-on-vaping-policies
[5] http://www.legislation.gov.uk/uksi/2005/1803/contents/made

evidence that they can be effective." Advice to UK women who are pregnant is that "..licensed nicotine replacement therapy (NRT) products such as patches and gum are the recommended option to help stop smoking. But if you find using an e-cigarette helpful for quitting and staying smoke free, this is much safer than continuing to smoke."[6]

21. Globally, regulation of E(N)NDS varies widely across countries and regions, with the spectrum ranging from an absence of regulation in some places through to complete prohibition in others (Brady et al. 2019).

Toxicological evaluation of E(N)NDS-related exposures

Particulate matter

22. Data on E(N)NDS aerosol PM were summarised in TOX/2017/49. Analyses of machine-produced aerosols have suggested that the particulate phase comprises submicron particles with a similar size distribution to CC smoke, as well as nanoparticles (< 100 nm). Studies of PM characteristics are difficult to compare due to the variability in test conditions and types of E(N)NDS products and liquids tested. The Committee supports the development of standardised and validated testing devices and protocols.

23. The Committee noted that it is not clear what effect manipulation of device power will have on particle characteristics. The particles may coalesce and any present as nanoparticles would be likely to agglomerate. Particles smaller than 10 μm would penetrate the airways and could physically affect the lung epithelium. Dilution undertaken as part of the studies would affect what is measured. The condensation rate of droplets is dependent on their concentration in air, so that the higher the concentration, the more rapidly they coalescence into larger droplet particles. Because of these effects, size distribution might not be key in determining the biological effect. The most relevant studies to humans would be those using low dilution and high humidity, and research studies should investigate deposited doses. It would be important to determine the solubility of the particles in the aerosol.

24. Solid particles, such as metal nanoparticles, have also been detected in E(N)NDS aerosols. The studies reviewed by COT used mostly first- or second-generation E(N)NDS devices (see TOX/2018/15). These studies indicated that metal particles are derived mostly from the E(N)NDS devices rather than the e-liquids and may leach into the e-liquid during storage. Concentrations of metal particles measured in aerosols varied widely both between and within brands. Reasons for this may include structural aspects of the E(N)NDS device, puffing protocols used in the study, variation in e-liquid components, and changes occurring with use and storage of products. Overall, the Committee concluded that there is likely to be some exposure to metals from use of E(N)NDS but there would need to be an appropriate comparison of such exposure with reference values. Caution is required in

[6] https://www.nhs.uk/live-well/quit-smoking/using-e-cigarettes-to-stop-smoking/

comparing data on exposures from E(N)NDS aerosol, which is intermittent in nature, with air quality guidelines (AQG) for metals, which assume 100% exposure over 24 hours. It would be helpful to compare levels of metals in aerosols with exposure from ambient concentrations as well as from CC and heated tobacco products in the future. Given the rate of development of E(N)NDS devices, it would be important to focus on more recent data; as device construction has changed over time, it might be expected that levels of some metals in E(N)NDS aerosols would have decreased. Details of the methodology used to determine the metal concentrations in the aerosol should be well documented.

25. Work by Williams et al. (2013) and Williams et al. (2017) found silicate fragments in E(N)NDS aerosol, which appeared to derive from the device sheath and wick (see TOX/2018/20). It was unclear whether the electron micrograph of a spherical amorphous silicate bead in the paper by Williams et al. (2013) was representative of the form of the majority of siliceous material present in the aerosol. For example, if silica were present in microcrystalline form, this would be of greater concern than if it were in amorphous form. In order to risk-assess E(N)NDS for their silicon/silicate content, there would be a requirement for further information on background exposure to inhaled silicates from ambient air, the form of the released material, and whether there were current engineering solutions that could minimise silicate release.

26. Data from studies reviewed by COT (TOX/2019/39) indicated that total particulate mass (TPM) measured in machine-produced E(N)NDS aerosols ranged up to a maximum of approximately 5000 µg/puff, depending on experimental conditions. In experimental studies, conducted in rooms or exposure chambers to evaluate ambient air PM levels in situations where E(N)NDS products were being used by human users (see TOX/2019/11), mean $PM_{2.5}$ levels increased in the range of approximately 150 to 1500 µg/m^3, depending on experimental conditions (including the number of users, usage time, air exchange rate, product type). Measurements taken *in situ* during one poorly ventilated indoor 'vaping event' indicated a mean ambient air PM_{10} concentration of approximately 8500 µg/m^3. These levels are generally much higher than the World Health Organisation (WHO) AQGs for $PM_{2.5}$ (25 µg/m^3 24 h mean; 10 µg/m^3 annual mean) and PM_{10} (50 µg/m^3 24 h mean; 20 µg/m^3 annual mean) (WHO 2006).

27. The Committee considered that it is unclear how applicable the WHO AQG would be to the assessment of E(N)NDS particulates. Relating to bystander exposure, the Committee noted that the solubility of PM from E(N)NDS is not clear, but most PM in E(N)NDS aerosol appears to be soluble (WHO, 2017) and hence its toxicological profile may well differ from that of insoluble particles (ECHA, 2017). Ambient air risk coefficients primarily relate to such insoluble particles but also include soluble constituents of atmospheric pollution, such as ammonium sulphate and ammonium nitrate. Overall, the Committee has some reservations over applying the risk coefficients for $PM_{2.5}$ in ambient air to E(N)NDS aerosol to estimate any potential health impacts. As no information on the lung deposition of PM from

E(N)NDS aerosol in animal models was available, the Committee considered that the PM is likely to comprise droplets of condensate and if it is primarily the E(N)NDS vehicle, the health-based guidance value (HBGV) for glycerol or PG could be used to assess the risk. The Committee concluded that there is significant uncertainty in the risk from PM in E(N)NDS aerosol. A further uncertainty is in the short-term nature of PM exposures arising from E(N)NDS use, for which good epidemiological data are not available to aid interpretation

Synthesis and COT opinions

- High levels of PM are present in the aerosols produced from E(N)NDS. This mostly comprises droplets that are formed when components within the E(N)NDS liquid are heated and vaporise, then condense back into liquid aerosol as the gas cools.

- E(N)NDS aerosols may also contain a small proportion of solid particles, likely to be derived from the structure of the E(N)NDS device. Given the wide variation in measured levels of metals emitted into E(N)NDS aerosols, both within and between brands, it is difficult to draw general conclusions on levels of exposure of users. As E(N)NDS devices do not all use the same materials, the presence and quantity of the different metals in E(N)NDS aerosols are likely to be related to the materials used in the construction of the particular device, and perhaps also to other factors, for example, build quality, use parameters and age of the device.

- There is substantial uncertainty in the risk from PM in E(N)NDS aerosol for users and bystanders. The issue has not been fully resolved, however soluble particles of otherwise low-toxicity substances might be less of a concern than insoluble ones if they were to disperse rapidly on deposition in the lung. The Committee considers that the combination of metals and other particles in E(N)NDS aerosol could exacerbate an inflammatory response. A further uncertainty is in the short-term nature of PM exposures arising from E(N)NDS use, for which good epidemiological data are not available to aid interpretation.

Propylene glycol

28. The toxicity of PG, within a context of relevance to use in E(N)NDS products, was reviewed in TOX/2018/19 and TOX/2018/23.

29. Toxicity associated with exposure to PG is extremely low. In rare cases, very high doses administered to humans, mostly by the intravenous (i.v.) route, have been reported to produce hyperosmolality, metabolic acidosis and renal dysfunction. PG is not considered to be genotoxic or carcinogenic. Acute exposure of humans to PG aerosol can cause eye and throat irritation, cough, nasal burning and stinging. However, evaluations of the possible effects of longer term exposures are limited by concomitant exposure to other substances. In animals, PG causes little or no skin

irritation, slight eye irritation and no skin sensitisation. The Committee considered that PG is not a skin sensitiser in humans. Experimental studies of exposure to inhaled PG aerosols in rats have shown local irritant effects. Given that the systemic half-life indicates that accumulation of PG would not occur, the Committee concluded that risk assessment should be based on potential adverse effects at the site of contact, namely the respiratory tract.

30. The key study was identified as that of Suber et al. (1989), in which rats exposed by inhalation to 160, 1010, or 2180 mg/m^3 PG aerosol six hours per day, five days per week, for thirteen weeks, showed nasal haemorrhaging and ocular discharge in all treatment groups (see TOX/2018/23). The study authors suggested that this effect was caused by local dehydration, but the Committee considered that an irritant effect was also possible. However, it was unusual for haemorrhage to have been reported for up to 13 weeks without any other pathology being observed. Microscopic evaluation of the nasal cavity had shown thickened respiratory epithelium in the posterior portion of the nasal cavity, with increased numbers of goblet cells and goblet cell mucin content in the medium- and high-dose groups. There were no histological changes in the trachea, lungs or larynx. The Committee considered that the nasal route of exposure is not directly relevant to use of E(N)NDS and noted that, given the reported particle diameter of approximately 2 µm in this study, the trachea and larynx would have been exposed and yet no adverse effects were observed in these tissues. However, while the route of exposure is of equivocal relevance, a lowest observed adverse effect concentration (LOAEC) of 160 mg/m^3 for nasal haemorrhaging could be used to protect against potential irritant effects at other sites of contact in the respiratory tract from E(N)NDS use. Using local dosimetry modelling, the LOAEC of 160 mg/m^3 in rats was extrapolated to a human equivalent concentration (HEC) for exposure to PG from E(N)NDS aerosol of 1650 mg/m^3 (see TOX/2020/29 and TOX/2020/29 Annex A for details of conversion from the LOAEC to the HEC).

31. Data from studies that measured levels of PG in E(N)NDS aerosols produced by machine puffing under controlled experimental conditions (see TOX/2019/39) indicated that one puff could contain a mean mass of approximately 0.7 mg PG (Kienhuis et al. 2015, Margham et al. 2016). Hence, the average daily concentration, based on a user taking 300 E(N)NDS puffs per day, was estimated to be 0.011 mg/L (11 mg/m^3)[7].

32. For risk assessment of PG, the Committee considered that an uncertainty factor (UF) or Margin of Exposure (MOE) of ≥ 10 to the HEC would indicate low concern. This margin would cover toxicodynamic differences between rats and humans, and inter-individual variations between humans. Interspecies toxicokinetic differences are taken into account in determining the HEC. While the HEC is derived from a LOAEC from the Suber et al. (1989) study, the nasal haemorrhaging was

[7] Concentration to which the user would be exposed averaged over 1 day, by multiplying by the number of puffs per day (300) and dividing by the daily tidal volume (16 breaths/min x 60 min x 24 h x 0.84 L/breath = 23,040 L).

observed at the LOAEC of 160 mg/m^3 in the absence of any other pathological findings at this exposure concentration, and therefore the Committee considered the use of a LOAEC to NOAEC adjustment factor was not necessary. This was supported by the evaluation of The Dutch Expert Committee on Occupational Standards which established a health-based occupational exposure limit for PG based on the study of Suber et al. (1989) where the dose of 160 mg/m^3 was considered to be a no observed adverse effect concentration (NOAEC) for increased numbers of goblet cells in male and female rats seen in the medium and high dose groups only (HCN 2007).

33. Using the average daily concentration of an E(N)NDS user of 11 mg/m^3 and the HEC of 1650 mg/m^3 results in an MOE of 150. The Committee considered this to indicate that repeated exposure of users to PG from E(N)NDS aerosol in the short to medium term would be of low concern. However, it was stressed that the long-term effects from repeated exposures are unknown.

34. To assess bystander exposure, the Committee considered experimental studies in which PG levels were measured in ambient air where E(N)NDS products were used (see TOX/2019/11). These studies indicated low-level increases during E(N)NDS use in PG concentration over baseline levels in ambient air. These increases ranged from 0.199 mg/m^3 in the study of Schober et al. (2014)[8] to 0.317 mg/m^3 in the study of Liu et al. (2017)[9]. The Committee considered that within the context of this evidence base, second-hand exposure to PG from E(N)NDS use would be unlikely to represent a concern for the health of bystanders.

Synthesis and COT opinions

- The available evidence base indicated low concern for the likelihood of adverse health effects in users from short to medium term exposure to PG from E(N)NDS. However, the effects of long-term repeated exposures are unknown.

- Based on the data set examined, exposure to PG in ambient air where E(N)NDS products are used is unlikely to represent a risk to bystanders.

Glycerol

35. The toxicity of glycerol, within a context of relevance to use in E(N)NDS products, was reviewed in TOX/2018/19 and TOX/2018/23. The glycerol in E(N)NDS liquids is often referred to as vegetable glycerine (VG).

36. Glycerol is an authorised food additive (E422) and is permitted in cosmetic products in the EU. Oral exposure to high levels is not usually associated with

[8] *ad libitum* product use by three users for two hours in a 45 m^3 room with an air exchange rate of 0.37-0.74 per hour

[9] a total of 1649 puffs taken by nine users over four hours in 114 m^3 exposure chamber, with fresh air supplied at a rate of 7.5 L/s

adverse effects. Glycerol is not considered to be genotoxic or carcinogenic. Glycerol may be slightly irritating to the eyes, not irritating to the skin and may have very slight skin sensitising potential in humans. Limited data are available regarding exposure to glycerol by inhalation.

37. The study by Renne (1992) was considered to provide the most suitable data for risk assessment (see TOX/2018/23). In this study, rats exposed to 33, 167, or 662 mg/m^3 glycerol aerosol six hours per day, five days per week, for thirteen weeks, showed no dose-related systemic effects. However, a statistically significant increased incidence of minimal-to-mild squamous metaplasia of the epiglottis in the highest-dose group was observed. The Committee noted that this area of the larynx is exposed to food particles and is very susceptible to squamous metaplasia. The rats were exposed nasally but no adverse effects were observed in the nasal passages as would be expected for an irritant. The Committee concluded that the squamous metaplasia was minimal and not of toxicological significance, and the top dose could be considered a NOAEC. From local dosimetry modelling studies, the NOAEC of 662 mg/m^3 in rats was extrapolated to a HEC for exposure to glycerol from E(N)NDS aerosol of 6280 mg/m^3 (see TOX/2020/29 and TOX/2020/29 Annex A for details of conversion from NOAEC to HEC).

38. Data from studies that measured levels of glycerol in E(N)NDS aerosols produced by machine puffing under controlled experimental conditions (see TOX/2019/39) indicated that one puff could contain a mean mass of approximately 1.6 mg glycerol (Margham et al. 2016). The average daily concentration, based on a user taking 300 E(N)NDS puffs per day, was estimated to be 0.025 mg/L (25 mg/m^3)[10].

39. For risk assessment of glycerol, the Committee considered that a UF or MOE of ≥ 10 to the HEC would be of low concern. This margin would cover toxicodynamic differences between rats and humans, and inter-individual variations between humans. Interspecies toxicokinetic differences were taken into account when determining the HEC.

40. Using the average daily concentration of an EN(N)DS user of 25 mg/m^3 and the HEC of 6820 mg/m^3 results in an MOE of 273. The Committee considered these findings to indicate that repeated exposure of users to glycerol from E(N)NDS aerosols in the short to medium term would be of low concern. However, it was emphasised that the long-term effects from repeated exposures are unknown.

41. To assess bystander exposure, the Committee considered experimental studies in which glycerol levels were measured in bystander air during E(N)NDS use (see TOX/2019/11). These studies indicated low-level increases during E(N)NDS use in glycerol concentration over baseline levels in ambient air. These increases

[10] Concentration to which the user would be exposed averaged over 1 day, by multiplying by the number of puffs per day (300) and dividing by the daily tidal volume (16 breaths/min x 60 min x 24 h x 0.84 L/breath = 23,040 L).

ranged from 0.73 mg/m^3 in the study of Schober et al. (2014)[11] to 0.242 mg/m^3 in the study of Liu et al. (2017)[12]. The Committee considered that within the context of this evidence base, second-hand exposure to glycerol from E(N)NDS use would be unlikely to represent a concern for the health of bystanders.

Synthesis and COT opinions

- The available evidence base indicated low concern for the likelihood of adverse health effects in users from short to medium term exposure to glycerol from E(N)NDS. However, the effects of long-term repeated exposures are unknown.

- Exposure to glycerol from E(N)NDS is unlikely to represent a risk to bystanders.

Nicotine

42. ENDS e-liquids contain nicotine as an aid to cessation of CC smoking. Regulation of ENDS in the UK is currently covered by the EU TPD, which specifies that e-liquids or nicotine refills may not contain nicotine at concentrations in excess of 20 mg/mL (see paragraph 16). However, in some other countries, for example the US, products may contain higher concentrations of nicotine. Measured e-liquid nicotine concentrations do not always reflect those listed on product labels. The yield for nicotine delivery from e-liquid to aerosol is not consistent across products and is affected by factors including e-liquid nicotine form and concentration, device power output, and puffing topography. Higher-power devices (generally newer-generation products) tend to be used with lower nicotine concentrations, as a result of more effective nicotine delivery. The Committee noted that using e-liquids with lower nicotine concentrations could, however, lead to increased exposure to other aerosol constituents, as users titrate to their desired nicotine intake. Nicotine absorption is pH dependent and so will be affected by the overall e-liquid formulation. The Committee acknowledged that, while its assessment covered as widely as possible the breadth of devices on which data were available, the continual fast-paced technological advances in E(N)NDS could affect nicotine exposures in ways not covered in this report.

43. Detailed reviews of nicotine pharmacokinetics can be found in Hukkanen et al. (2005) and Benowitz et al. (2009). The kinetics of nicotine delivery vary with the mode of administration. Pulmonary absorption is rapid and this has been associated with the dependence effects of nicotine via CC smoking. Absorption from the mouth and upper respiratory tract is slower. Blood plasma nicotine levels in CC smokers generally range from 10 to 50 ng/mL, with typical daily trough concentrations of 10 to 37 ng/mL and peaks of 19 to 50 ng/mL, and a mean nicotine boost per 1 CC smoked

[11] *ad libitum* use by three users for two hours in a 45 m^3 room, with an air exchange rate of 0.37-0.74 per hour

[12] a total of 1649 puffs taken by nine users over four hours in a 114 m^3 exposure chamber, with fresh air supplied at a rate of 7.5 L/s

of 10.9 ng/mL. Chronic nicotine exposure is often associated with the development of addiction. Daily ENDS usage patterns vary but long-term systemic exposure to nicotine is expected to occur, with the intended use to aid smoking cessation. Clinical studies of the pharmacokinetics of nicotine from ENDS were reviewed in the National Academy of Sciences publication 'Public Health Consequences of E-Cigarettes' (Chapter 4: Nicotine) (NAS 2018). Studies indicated that the level of systemic nicotine exposure from ENDS is dependent on user and usage profile, device power, and e-liquid nicotine concentration. Inexperienced ENDS users often achieve only low levels of nicotine delivery, but with greater experience users develop the ability to extract more nicotine and can achieve levels of nicotine delivery and systemic retention similar to those of CC smoking. Studies that used ENDS devices with high power or high nicotine concentration in the e-liquids showed that plasma nicotine concentrations after short puffing bouts (e.g. 10-15 puffs) were in the same range as those achieved from CC smoking.

44. Biomonitoring studies have assessed nicotine exposure in E(N)NDS users (see TOX/2019/39) and in bystanders (see TOX/2019/11). Studies of people switching from CC to ENDS have produced variable findings relating to plasma nicotine concentrations achieved by users, while studies of long-term ENDS users have indicated plasma nicotine levels similar to those in regular CC smokers. In bystanders, a small data set (three studies) reporting biomonitoring indicated higher serum cotinine levels in bystanders exposed to ambient air where ENDS were used compared with non-exposed control subjects.

45. The Committee noted that the similar pharmacokinetic profile for nicotine from a modern ENDS device and a CC may be desirable in using ENDS as a smoking cessation aid. However, this might also result in addiction to nicotine in naïve users. But addiction to CC is complex and the addiction process involves factors in addition to nicotine itself, so that addiction in ENDS users may differ is some ways from that in CC smokers.

46. The COT reviewed general toxicological data on nicotine (see TOX/2019/38). Nicotine is acutely toxic via all routes of exposure, targeting the central and peripheral nervous systems. In humans, the lethal dose is widely cited at approximately 0.6–1.0 mg/kg bw, based on historical reports of poisoning, although this was challenged in a recent review of the literature which suggested that the lethal dose is in the range of 6.5–13 mg/kg bw (Mayer 2014). Poisoning cases mostly relate to accidental or deliberate ingestion or dermal exposure. Nicotine is reported to cause local irritation at the site of administration (e.g. dermal patch, nasal or oral sprays) in humans. Nicotine has acute effects on the cardiovascular system, including increased heart rate and blood pressure, and adverse effects on the respiratory system, including suppression of cough and mucociliary clearance in humans, and increased airways hyper-responsiveness and impaired mucociliary clearance in animals. No data were identified regarding repeated or long-term inhalation exposure to nicotine *per se* in humans and data on longer term effects of nicotine exposure from ENDS are not currently available. Some evaluations have

been made based on data from studies of NRT as an aid to quit CC smoking. On cardiovascular disease, these evaluations have concluded that studies are mostly of inadequate quality to draw clear conclusions but have not shown evidence of serious cardiovascular events. The Lung Health Study reported by Murray et al. (2009) found an absence of any relationship between NRT use and lung, gastrointestinal, or all cancers over a relatively short follow-up period (7.5 years).

47. The COT reviewed literature on possible effects of exposure to nicotine on developmental outcomes in humans, from exposure via the parents prior to or during pregnancy or lactation (TOX/2018/45) and in adolescents and young adults (TOX/2019/01). A few studies had reported a potential association between prescription of NRT during pregnancy and adverse birth outcomes. However, as the available studies on NRT showed only low levels of abstinence from smoking, the source of nicotine (NRT or tobacco) cannot be determined. This reduces confidence that the effects observed were nicotine specific, although it is also noted that this would reflect the real world. In addition, the studies were not designed to investigate the health effects of nicotine. No data were available on direct effects of nicotine exposure in human adolescents, so the evidence in the literature was drawn from data on CC, which expose the user to a range of potentially toxic substances in addition to nicotine, such as particulates, carbon monoxide and other vasoactive substances, which may not be present in ENDS aerosol, or are present at lower levels. Brain development continues until around 25 years of age in humans. This is important, as cognitive development is still occurring during adolescence and nicotine is neuroactive. Hence, adverse neurodevelopmental effects might occur, though more information on the quantification of these potential effects is required. There is also concern about the possible relationship between nicotine use and anxiety, depression and other neuro-psychiatric and neuro-functional effects, and it is not known whether ENDS could have similar effects.

48. Animal studies on the effects of nicotine appeared to mirror effects seen in human studies following CC exposure. Most studies administered nicotine via non-inhalation routes, with exposures intended to model the systemic nicotine exposures that would be achieved via direct CC smoking or from second-hand smoke exposure. Findings have indicated that nicotine can have negative effects on brain development. Effects can be subtle, expressed during specific life periods, and persist into adulthood. Nicotine can also adversely affect fetal airway development and lung histology. A comprehensive review of this area can be found in England et al. (2017). The Committee concluded that taking into consideration the effects observed in animal studies, particularly on the developing lungs, there is good biological plausibility for an effect of nicotine on development. However, the Committee has reservations about trying to quantify the effects of nicotine in humans from the animal studies as the relationship of the dosing to human exposures is not clear.

49. The Committee noted that case reports have described poisonings from ENDS containing nicotine. Effects have included vomiting, lactic acidosis, and in

some cases, death. Many cases relate to ingestion of e-liquids by young children. Atypical exposure to nicotine, for example accidentally inhaling e-liquid due to device failure or mistaking the liquid for another product such as eye drops, could be associated with toxicity.

50. In the evidence base considered by the COT, there was a paucity of suitable data which could be used as a basis to establish an inhalation HBGV for nicotine. The study reported by Lindgren et al. (1999) was considered to be the most suitable from which to establish an HBGV for nicotine exposure in people switching to ENDS from CC smoking (TOX/2019/47, TOX/2019/72). In this study, heart rate (HR) and electroencephalographic (EEG) parameters were monitored in human CC smokers administered nicotine at doses of 0, 3.5, 7.0, 14.0, and 28.0 µg/kg bw by i.v. infusion over a 10-minute period, following a 12-hour abstinence from smoking. The Committee considered that the data on HR changes presented in the publication showed no clear effect at the lower doses but were inadequate to determine a point of departure (POD) (the study was not specifically designed to investigate effects on HR). However, a NOAEL of 7 µg/kg bw could be identified for changes on EEG, albeit with some uncertainty. An adjustment of 0.55 was used to extrapolate from i.v. to inhalation route[13]. An UF of 5 was used to account for human variability, as the effect was C_{max}-dependent and hence would be subject to less toxicokinetic variability than AUC-dependent effects. Using these values, an HBGV of 2.5 µg/kg bw was established for acute inhalation exposure to nicotine of users switching to ENDS from CC smoking. Given that the endpoint is a sensitive pharmacological effect, the HBGV was also considered to be adequate to protect against longer-term effects.

51. In the studies summarised in TOX/2019/39 that measured levels of nicotine in E(N)NDS aerosols produced by machine puffing under controlled experimental conditions, the highest mean aerosol nicotine content was 43 µg/puff (range 18-93 µg/puff) produced from a range of ENDS products with measured nicotine concentrations of 11.5–27.4 mg/mL in the e-liquid (Laugesen 2015). Based on these data, for a 70 kg user taking 15 puffs during one ENDS-use session[14], average (range) exposure would be 9.2 (3.9-19.9) µg/kg bw. This would exceed the COT HBGV by approximately four-fold. Calculating daily nicotine exposure using data on estimated average daily ENDS use from the study of Dawkins et al. (2018) (see paragraph 7), for a 70 kg user taking 272 or 338 puffs/day, average (range) daily exposure would be 167 (70-361) or 208 (87-449) µg/kg bw/day, respectively. This would exceed the COT HBGV by approximately 70- to 80-fold.

52. An average daily exposure to nicotine from smoking CC has been reported as 500 µg/kg bw/day (*data taken from* Benowitz and Jacob (1984), see TOX/2019/39). This level of nicotine exposure exceeds the COT HBGV by approximately 200-fold. The estimated mean levels of daily exposure to nicotine from ENDS use calculated

[13] Estimate taken from data presented in Hukkanen et al. (2005).
[14] 15 puffs is suggested as a possible scenario for one ENDS-use session, although it is acknowledged that patterns of use vary between users.

in paragraph 51 represent approximately 33–42% of this average daily exposure from CC smoking.

53. The Committee considered that the HBGV for nicotine of 2.5 µg/kg bw/day calculated from the study of Lindgren et al. (1999) was not suitable for risk assessment in previously nicotine-naïve ENDS users or in bystanders, as the study had been conducted in CC smokers after 12-hour abstinence from smoking and there was some evidence that a different level of effect would be expected in people who were not nicotine users. The Committee agreed that analyses for these two groups could be conducted with the NOAEL identified in the Lindgren et al (1999) study, using a margin of exposure (MOE) approach. From literature reporting clinical studies comparing acute effects of nicotine exposure in non-smokers (summarised in TOX/2019/72), a sensitivity analysis suggested that non-smokers may be approximately three-fold more sensitive to the acute effects of nicotine than CC smokers. The Committee therefore agreed that an additional factor of 3 should be used with the margin of 5 determined in para 50 above, to account for human variability. Hence, an MOE of ≥ 15 would indicate low concern for risks from nicotine in nicotine-naïve individuals.

54. Using the exposure data in paragraph 51 and the NOAEL of 12.7 µg/kg bw (adjusting by 0.55 for i.v. to inhalation exposure as explained in para 50), for nicotine-naïve users taking up ENDS (assuming 70 kg body weight), the mean (range) MOE values for nicotine exposure would be 1.38 (3.26-0.64) for a 15-puff ENDS-use session, 0.076 (0.181-0.035) for 272 puffs/day, and 0.061 (0.146-0.028) for 338 puffs/day[15]. These MOE values are substantially below the indicative MOE of 15, and therefore, it is anticipated that nicotine-naïve individuals may experience effects from nicotine on first use of ENDS.

55. For bystanders, 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) µg/m^3, reported by Czogala et al. (2014). In this study, one individual used an ENDS product containing 16–18 mg/mL nicotine *ad libitum* for two 5-minute periods with a 30-minute interval, in a 39 m^3 chamber (the air-exchange rate in the chamber was not specified). Based on these data, for a 70 kg individual inhaling 20 m^3 air during 24 hours this would lead to a nicotine intake of 0.95 (0.19-1.78) µg/kg bw/day, or for a 13.3 kg, 1-6 year-old child inhaling 8.8 m^3 air during 24 hours this would lead to a nicotine intake of 2.2 (0.43-4.1) µg/kg bw/day. Using these exposure data and the NOAEL of 12.7 µg/kg bw as in para 54, the MOE values for daily nicotine exposure of bystander adults and 1-6 year-old children are 13.4 (66.8-7.13) and 5.77 (29.5-3.10), respectively. Some of these MOE values are below the indicative MOE of 15,

[15] These evaluations are not intended to be regarded as specific analyses of use of certain products or scenarios, but rather have been carried out in order to provide a general representation of possible margins of exposure for nicotine exposure from ENDS.

so it is possible that some bystanders will experience effects from nicotine under these exposure scenarios.

56. 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. 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. In homes where CC smoking occurred, average nicotine levels were often in the range of 1–3 $\mu g/m^3$, with higher ranges measured during active smoking (e.g. 5–15 $\mu g/m^3$). Workplace studies showed a wide range of nicotine concentrations, with mean levels often in the range of 1–10 $\mu g/m^3$ but ranging up to around 50 $\mu g/m^3$ where smoking was allowed, with levels generally < 1 $\mu g/m^3$ 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 $\mu g/m^3$. A study of waiters exposed to second-hand smoke showed average nicotine levels of 5.8 $\mu g/m^3$, with an upper range of 68 $\mu g/m^3$, while a study in a cafeteria showed nicotine concentrations of 25-40 $\mu g/m^3$ in a smoking section, 2–5 $\mu g/m^3$ in a proximal non-smoking section, and < 0.5 $\mu g/m^3$ in a more-distant non-smoking section. Nicotine levels in bars and lounges were generally > 10 $\mu g/m^3$ and often > 50 $\mu g/m^3$, with maximum levels > 100 $\mu g/m^3$ occasionally noted in bars (CDC 2006).

57. The mean nicotine level of 3.32 $\mu g/m^3$ measured by Czogala et al. (2014) (see paragraph 55), associated with moderate use of an ENDS product by one user in a 39 m^3 chamber for two 5-minute periods over one hour, is within a similar range as that described in the U.S. Surgeon General report for levels of nicotine in households where CC smoking takes place.

58. Two publications listed in TOX/2019/11 reported nicotine levels measured in ambient air during indoor 'vaping events'. Johnson et al. (2018) reported a median nicotine level of 1.1 $\mu g/m^3$ from measurements taken during four vaping events held in well-ventilated venues in South-Eastern US. However, Chen et al. (2017) measured a mean ambient air nicotine concentration of 124.7 $\mu g/m^3$ during a 'vaping event' held in a poorly ventilated convention centre in Maryland, US. The concentration of 124.7 $\mu g/m^3$ 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.

59. The Committee considered that using ENDS to replace smoking could potentially lower risks related to CC smoking. However, overall, existing nicotine users will continue to have the same risks to themselves and their offspring from exposure to a given level of nicotine as they would have through use of CC. Non-users who have never been exposed to nicotine and take up vaping will be at risk from toxicological effects of nicotine to which they would not otherwise be exposed. An additional factor in this assessment is the extent to which naïve users

take up ENDS rather than CC. This is an aspect on which the Committee does not have the expertise to comment. For bystanders, studies have indicated that nicotine is emitted into ambient air from ENDS use and this may be associated with increased risk of nicotine-related adverse health effects in some bystanders. In areas where smoking is not permitted, this could result in a risk from nicotine exposure to which they would not otherwise be subject.

60. The Committee also noted that use of ENDS while continuing to smoke CC could potentially lead to increased nicotine exposure compared with that from CC smoking only. The Committee concluded that dual use of CC and ENDS would not necessarily reduce the risk of adverse health effects associated with CC smoking overall, and may even increase the overall risk.

Synthesis and COT opinions

- Experienced users self-titrate nicotine intake from ENDS. Systemic exposure levels of nicotine equivalent to those from CC smoking can be achieved. Factors influencing the level of nicotine exposure and retention include ENDS product type, user profile, usage parameters, e-liquid nicotine concentration, and the overall formulation of the e-liquid.

- For people who switch from CC smoking, the risks associated with nicotine exposure from ENDS would be expected to be similar to those from the same nicotine exposures through use of CC.

- It is thus anticipated that nicotine-related health effects could occur with long-term use of ENDS. Risks include effects on a large range of endpoints in users and their offspring.

- Non-users who have never been exposed to nicotine and who take up vaping would be at risk from effects of nicotine to which they would not otherwise be exposed. This also includes the risk of addiction.

- Use of ENDS while continuing to smoke CC (dual use) could potentially lead to increased nicotine exposure compared with that from CC smoking only, and may increase the overall risk.

- Bystanders are likely to be exposed to some nicotine in ambient air where ENDS products are used, which may have some associated effects.

Flavourings and their degradation products

61. E(N)NDS liquids with thousands of unique flavour-names have been listed. Although many of the flavouring additives are substances that have been assessed for use in foods, few have undergone acute or chronic toxicity testing via the inhalation route. Two areas of concern were noted with respect to the potential toxicity of E(N)NDS flavouring compounds. Firstly, there is the potential for systemic toxicity, which would likely be covered through information on oral toxicity, although

the effects of heating the flavouring in the E(N)NDS device would need to be considered. The other aspect is route-specific toxicity, including local effects.

62. The Committee agreed an approach could be adopted considering the toxicity assessment conducted for use of flavouring compounds as food additives, adding in consideration of whether there would be any potential specific effect associated with inhalation exposure or as a result of heating in an E(N)NDS device. This would be preferable to requiring a full toxicity data package and the potential for unnecessary toxicity studies to be carried out (see Annex B).

63. The Committee reviewed toxicological data for four of the principal flavouring compounds used in e-liquids, namely menthol (TOX/2019/48), vanillin (TOX/2019/24), cinnamaldehyde (TOX/2019/25), and menthone (TOX/2019/58).

64. **Menthol** has been classified under CLP as a skin and eye irritant as it induced irritation effects in experimental studies. It may also be a respiratory irritant following inhalation, as inflammation of upper respiratory tissues, rhinitis, lacrimation and ocular redness were reported by some employees exposed to up to 39.4 mg/m^3 menthol. The respiratory sensory irritation potential of menthol has been investigated using a number of approaches. The RD50 was measured in mice and also calculated based on physico-chemical parameters. Menthol is known to activate transient receptor potential melastatin member 8 (TRPM8), which is responsible for its cooling, analgesic and counter-irritant properties; this may also affect the sensory impact of nicotine and irritants in E(N)NDS aerosol. Menthol is not considered to be mutagenic. Epidemiology data show that mentholation of cigarettes has no effect on the lung carcinogenicity of cigarettes. In addition, experimental data, via the oral and dermal routes, did not show evidence of carcinogenicity. A number of repeat dose studies have been carried out in experimental animals that assessed either exposure to menthol or to cigarette smoke containing menthol. No adverse effects were observed that could be attributed to menthol. No reproductive or developmental studies could be identified that addressed the impact of menthol via the inhalation route on these endpoints. The Committee considered that menthol-related bronchodilation, antitussive effects, decreased inhalation rate, and mucus production are likely to be due to irritant effects as they are related to the activation of TRPM8. There are a number of uncertainties regarding the potential for menthol to increase the risk of infection or action of irritants in the open airways and the extent of its effect(s) on lung clearance; the relevance of the formation of metabolites and breakdown products of menthol at high temperatures and whether these are different from degradation products formed in cooked foods; and differences in metabolism following oral exposure to menthol compared to inhalation exposure.

65. Data suggested that **vanillin** may act as a sensory irritant in E(N)NDS users. Some skin irritation was reported in animals although it is unclear if they were exposed to vanillin, or 4-methoxy-benzaldehyde in a read-across approach. Eye irritation was also reported but was due to the mechanical effect of the crystals. There is some evidence for skin sensitisation in humans, but studies in animals

indicate only weak sensitisation potential. Although mixed results were obtained regarding the mutagenicity of vanillin, overall, it was not considered to be mutagenic. No repeat dose, reproductive or carcinogenicity studies carried out with vanillin via the inhalation route were identified. The Committee noted that there was potential concern over acetal formation with PG or glycerol and vanillin in E(N)NDS aerosols, which could occur at room temperature. The Committee considered that as these chemicals are present in foods, acetal formation might also occur in food, in which case no specific assessment of systemic toxicity of acetal would be required. Extrapolation from food where flavourings could be cooked might be appropriate, although consideration would need to be given to systemic exposure levels of the degradation products by the different routes. For vanillin itself, read across from 4-methoxy-benzaldehyde was considered reasonable, though with respect to acute toxicity, it was considered that exposure from ENDS in any case was unlikely to be of concern.

66. Data indicated that **cinnamaldehyde** may act as an airway irritant in E(N)NDS users. Cinnamaldehyde caused skin and eye irritation in animals and humans. There was convincing evidence for skin sensitisation, which may indicate a potential for respiratory sensitisation, and the Committee agreed that this was of concern. Some positive results were obtained in *in vitro* mutagenicity tests. However, these were not replicated *in vivo*. Overall, cinnamaldehyde was not considered to be mutagenic. No repeat dose, reproductive or carcinogenicity studies carried out with cinnamaldehyde via the inhalation route were identified.

67. The availability of data regarding inhalation toxicity of **menthone** was limited. No data were available regarding acute toxicity or reproductive and developmental toxicity. There was no information on thermal decomposition of menthone. The literature indicated that menthone was not considered to be mutagenic. The Committee concluded that overall there is a large data gap regarding repeat dose inhalation toxicity for menthone.

68. From discussions on toxicological aspects of flavourings used in E(N)NDS products, the Committee agreed that assessment should be carried out using a framework for risk assessment of flavouring compounds via inhalation exposure. The Committee published its framework in COT Statement 2020/01, which is attached at Annex B, along with worked examples for the four flavouring compounds considered in this section.

69. A number of data gaps with respect to E(N)NDS flavourings in general were identified. Information is required on which flavourings are used most commonly in E(N)NDS products, but these data are not readily available for the UK. Other gaps include the potential for co-exposure to flavouring compounds, either within a single e-liquid or resulting from the common practice of mixing e-liquids. Knowledge on effects of flavouring compounds on e-liquid pH would be important, as this can affect nicotine exposure. There also remains some uncertainty about the temperature to

which e-liquids may be heated on E(N)NDS use, and to what extent this may be affected by customisation of E(N)NDS devices by individual users.

Synthesis and COT opinions

- Flavourings should be suitably assessed for potential inhalation toxicity, as safety for use in E(N)NDS cannot be assumed based on their safety for use in food.

- There are a number of data gaps regarding potential toxicity of inhalation exposure to flavouring chemicals. For example, the potential to form new compounds of toxicological concern during heating or degradation including in the presence of e-liquid solvents should be considered.

- The COT has drawn up a framework to aid risk assessment of flavourings. Due consideration also needs to be given to any regulatory requirements at the time of product notification.

- Menthol, vanillin, cinnamaldehyde and menthone are commonly used flavouring compounds in e-liquids. Menthol is a skin and eye irritant and may also be a respiratory local and sensory irritant following inhalation. Vanillin may be a sensory irritant. Cinnamaldehyde is a respiratory irritant and there is concern over the potential for sensitisation. There is a large data gap regarding the inhalation toxicity of menthone.

Other constituents and products formed on aerosolization

70. Data reported from analytical studies of e-liquids and aerosols were collated in TOX/2018/16, TOX/2019/39, and TOX/2019/58.

71. Contaminants and/or impurities that have been detected in some e-liquids, often at low or trace levels, include nicotine-related contaminants (minor tobacco alkaloids and tobacco-specific nitrosamines (TSNAs)), metals and silicates (discussed in paragraphs 24-25), ethylene glycol, diethyl phthalate and diethylhexyl phthalate, ethanol, and traces of microbial toxins[16]. In some cases, testing of individual e-liquid samples has revealed the presence of active compounds such as synthetic cannabinoids. The Committee noted that the potential for the presence in e-liquids of drugs that are illegal in the UK is a cause for concern. However, the reports that were identified related to products that were marketed outside of the UK. Abuse and misuse patterns vary between different countries, and contamination in non-UK countries is outside the scope of the current review. Several studies have reported e-liquid samples that are not true-to-label. The Committee highlighted the need for e-liquid contents to be derived from reputable sources.

72. Contaminants and impurities in e-liquids might be transferred to aerosol on puffing. The few studies that measured TSNAs generally found that levels of NNN,

[16] The scope of the COT review did not include evaluation of non-chemical contaminants.

NNK, NAT, and NAB[17] were below the limit of detection (LOD) or limit of quantitation (LOQ) for the analytical method used or were quantifiable but very low. In the study by Goniewicz et al. (2014), NNN and NNK were, in each case, identified in nine out of the twelve samples tested, with highest reported individual readings of 0.029 ng/puff for NNN and 0.189 ng/puff for NNK. In a study reported by Margham et al. (2016), NNN was detected at 0.054 ng/puff. Tayyarah and Long (2014) reported that one of the five product types tested produced aerosol with a quantifiable content of NAT (0.2 ng/puff). Studies that also provided comparative data indicated TSNA levels in CC smoke in the ranges of 7.93-25 ng/puff for NNN, 10.12-26.7 ng/puff for NNK, 9.76-25.4 ng/puff for NAT, and 1.22-2.85 ng/puff for NAB. The Committee considered that available data indicated that exposure to TSNAs from E(N)NDS aerosols is likely to be very low.

73. A few studies reported the detection of volatile organic compounds (VOCs), e.g. benzene or toluene, and polycyclic aromatic hydrocarbons (PAHs) in E(N)NDS emissions, but the levels detected were generally very low.

74. In addition to transmission of constituents from e-liquid to aerosol, the vaping process itself may lead to formation and release into aerosol of chemical species that are not present in e-liquid. Thermal decomposition of e-liquids during aerosol production can form degradation products such as the carbonyl compounds, formaldehyde, acetaldehyde, and acrolein. Levels reported range widely. The extent to which thermal breakdown occurs is likely to be related to user puffing parameters and the operating characteristics of the E(N)NDS device, such as battery output and heating-coil resistance, which affect the temperature attained. This is an area of debate, with some commentators asserting that carbonyl production occurs only during ‘dry puffing’ (in the absence of e-liquid), which would be avoided by E(N)NDS users due to the disagreeable experience, while others have suggested that standard methods of analysis underestimate levels of carbonyl emissions into aerosol. The method by which the e-liquid is applied to the heating coil has also been reported to affect levels of degradation products in the aerosol. In most E(N)NDS apparatus, liquid is drawn to the coil through a wick. However, some devices allow ‘direct dripping’ of liquid onto the heating element, which appears to be associated with substantially increased levels of carbonyl emissions.

75. In the analytical studies that measured carbonyl emissions in aerosol produced by machine puffing (and excluding studies using atypical methods such as direct dripping), concentrations ranged from non-detectable up to highest average levels of 1940 mg/m^3 for formaldehyde, 1000 mg/m^3 for acetaldehyde, and 420 mg/m^3 for acrolein. These highest levels were reported from the study of Sleiman et al. (2016), in which a refillable tank-style (eGo) device containing a tobacco-flavoured e-liquid (50:50 PG:glycerol + 18 mg/mL nicotine) was operated at 4.8 V / 2.6 ohms. These upper levels exceed available international HBGVs for inhalation exposure to these chemicals by several orders of magnitude. However,

[17] NNN: *N*-nitrosonornicotine, NNK: nicotine-derived nitrosamine ketone, NAB: *N*-nitrosoanabasine, NAT: *N*-nitrosoanatabasine

the assessment is limited by the difference in the exposure scenario (intermittent short peaks) from that on which the HBGVs are based, i.e. continuous exposure. Additionally, it is debatable whether the high levels of carbonyl degradation products detected in some analytical studies of E(N)NDS aerosols produced by machine puffing would actually occur during normal use of E(N)NDS products by users.

76. Studies have also investigated biomarkers of exposure to tobacco-related toxicants associated with E(N)NDS use, in comparison with levels in users of other tobacco products and in non-users of tobacco products. The evidence base included short-term clinical studies and cross-sectional epidemiological studies. The majority of reports noted that biomarkers of exposure to tobacco-related toxicants are lower from E(N)NDS use than from CC smoking, but the data that were available did not indicate that levels are as low as those measured in non-users of tobacco products (TOX/2019/39).

77. Within the extremely limited data set that was available for assessment of contaminants or degradation products in ambient air where E(N)NDS products are used, there was no indication of any harmful emissions at levels of concern. No biomonitoring data were available for bystanders (TOX/2019/11).

Synthesis and COT opinions

- E-liquids may contain substances other than the standard contents necessary for the intended use of E(N)NDS as an aid to CC smoking cessation. These can include contaminants and impurities derived from the e-liquid formulants, and other substances that have been added to the e-liquid. These substances may, to some extent, be transferred to aerosol and inhaled by the user. E-liquid contents should be derived from reputable sources.

- Under some conditions of use, e-liquid contents have been shown to undergo thermal breakdown leading to the presence of toxic degradation products such as formaldehyde in the aerosol. The extent to which this would occur during normal use of E(N)NDS is unclear. Exposures from the inhaled aerosols in these studies would be above recommended air concentrations, but the short and intermittent pattern of exposure makes it difficult to make a robust assessment of any potential risk.

- Data from biomonitoring studies support the conclusion that exposure to levels of tobacco-related toxicants associated with E(N)NDS use is lower than from CC smoking, but not a low as in non-users of tobacco products. Very limited data on such exposures were identified for E(N)NDS bystanders.

Toxicity of the E(N)NDS aerosol

78. The COT reviewed published data relevant to the toxicity of E(N)NDS aerosol as a mixture (TOX/2018/24 and TOX/2018/46).

79. Human and animal studies that focussed on a range of respiratory and cardiovascular endpoints were reviewed in TOX/2018/24. The Committee considered that there were no unexpected findings and case reports of adverse conditions that appeared to be related to E(N)NDS use did not provide evidence for any cause and effect relationships above what would be expected from the inhalation of vapour containing nicotine.

80. The Committee considered that the effects of nicotine observed in CC smokers would also be expected to be observed in ENDS users. It was difficult to extrapolate from experimental studies using E(N)NDS aerosols due to the great variation in the aerosol as a whole. Use of E(N)NDS by adolescents prone to asthma resulted in exacerbation of symptoms, but the magnitude of this effect was not greater than would be expected from CC smoking. Data evaluated on non-neurological effects of E(N)NDS suggested that no such effects would occur just in adolescents as compared with adults. The Committee noted the potential for allergic or other health effects arising from exposure to E(N)NDS aerosol.

81. A small number of studies that had investigated potential developmental toxicity of E(N)NDS aerosols in animal models (see TOX/2018/46) supported the conclusion of effects of nicotine on development of the neurological and respiratory systems (see paragraph 48). However, it was not possible to quantify relative risks for developmental toxicity, nor to conclude specifically that nicotine was the sole contributing factor in these studies.

82. Advice was sought from the Committee on Mutagenicity (COM) and Committee on Carcinogenicity (COC) on the absolute and relative genotoxicity and carcinogenicity risks of E(N)NDS compared with CC and, if possible, heated tobacco products. Papers on these topics were discussed at the June 2018 (COM) and July 2018 and November 2019 (COC) meetings. The COC concluded that relative risk of E(N)NDS compared to conventional cigarettes appeared to be lower, but there was still some risk associated with the chemicals and particles in the emissions from E(N)NDS. This risk should be emphasised to new users. The COC considered that nicotine is not a carcinogen. The COM conclusions indicated a lack of consistency in the evidence base depending on the type of study.

83. Overall the COT agreed that the evidence on the toxicity of E(N)NDS aerosol indicates that use of E(N)NDS products may be associated with a reduced risk compared with CC, but this should not be taken as meaning that these products are risk-free. There is not, as yet, sufficient information of the consequences of long-term exposure, and such studies would help to identify any vulnerable individuals. Naïve users of E(N)NDS products who have respiratory sensitivity could plausibly be susceptible, and there would be particular concern for such users with chronic obstructive pulmonary disease (COPD), asthma, cardiovascular disease and cystic fibrosis as susceptible groups. The Yellow Card Scheme reporting of experiences of adverse effects will to some extent allow more data capture.

Synthesis and COT opinions

- It is difficult to extrapolate from the findings of studies involving E(N)NDS aerosol mixtures in experimental studies due to the wide range of variation in the mixtures tested, and devices and protocols used for aerosol production.

- In general, findings indicate that pharmacological effects related to nicotine exposure occur but these effects are not greater than would be expected via exposure to nicotine from CC.

- A limited available evidence base of studies in animal models supports the conclusion that exposure to ENDS aerosols may be associated with adverse developmental effects on the neurological and respiratory systems. This may be due to nicotine, although this cannot be concluded unequivocally from the available data.

- There is a lack of information regarding effects of long-term exposure to E(N)NDS aerosols. Prospective epidemiological studies would help to address this data gap.

Summary of the COT review of E(N)NDS

84. The COT reviewed the potential toxicological risks from E(N)NDS. The main perspective of the review was to evaluate the risks associated with intended use as an aid to CC smoking cessation. The assessment for users was predominantly limited to effects in adults, as ENDS products are not permitted for sale to anyone under 18 years of age in the UK[18]. For bystanders, the assessment covered all groups. Individuals were considered broadly to fall into one of the following three exposure scenarios:

- CC smokers switching to E(N)NDS as an aid to smoking cessation. For these individuals, the Committee considered that it was important to assess both relative risk from E(N)NDS use as compared with CC smoking and the absolute risk associated with use of E(N)NDS *per se*. Dual use may occur in the situation where people do not make a complete switch from CC to E(N)NDS.

- Non-users of tobacco products who take up E(N)NDS use *de novo*. In this case, the absolute risk would be important. However, the Committee noted that people who take up use of E(N)NDS may otherwise have taken up CC smoking. It is also possible that people in this group may progress from E(N)NDS use to CC smoking.

[18] Except for sales of medicines and medical devices in accordance with a prescription, in line with The Nicotine Inhaling Products (Age of Sale and Proxy Purchasing) Regulations 2015 (https://www.legislation.gov.uk/uksi/2015/895/contents/made)

- Bystanders, for whom it would be important to assess the absolute risk of exposure to E(N)NDS emissions in ambient air.

85. The reported contents and constituents of e-liquids and E(N)NDS aerosols were summarised and toxicological data were reviewed. Particulate matter and nicotine were identified as two principal exposures deriving from normal use of E(N)NDS that may potentially be associated with adverse health effects. Inhalation of flavouring ingredients is an area of particular uncertainty. This is a difficult aspect to address due to the large number of possible flavouring compounds used and the lack of toxicological data relating to inhalation exposure. A COT framework for risk assessment of flavouring compounds via inhalation exposure has been published. Potential contaminants and impurities of concern include metal particles, which may leach into e-liquids from E(N)NDS devices, and silicates. Breakdown of e-liquid contents during aerosolization can result in the formation of degradation products such as formaldehyde or other carbonyl compounds, although the extent to which this would occur during normal use of E(N)NDS by users has not been established. Breakdown products of e-liquid solvents and flavouring compounds could combine to produce species of toxicological concern. Data on this latter aspect are also lacking.

86. In considering the comparison of E(N)NDS use with CC smoking, the Committee concluded that the relative risk of adverse health effects would be expected to be substantially lower from E(N)NDS. This risk reduction would occur if people who are already smoking CC switch to E(N)NDS, or if E(N)NDS are taken up instead of CC. This is supported by biomonitoring studies which show lower levels of tobacco-related toxicants in E(N)NDS users compared with CC smokers. However, the reduction in risk would depend on the endpoint considered. A considerable reduction in risk of lung cancer would be anticipated due to lower exposure to tobacco-related carcinogens, but this would not necessarily be the case for all endpoints. The expectation of lower risk associated with E(N)NDS use compared with CC smoking relates to individuals making a complete switch from CC smoking to E(N)NDS. Although the Committee did not consider in detail the potential risks relating to dual use of E(N)NDS and CC, there is some evidence that dual use could lead to increased risk compared with CC smoking only, depending on use patterns. Pharmacokinetic studies have indicated that systemic exposure to nicotine from the types of ENDS products that have been studied to date is lower than or equivalent to that from CC smoking, but generally not higher. Therefore, any toxicological risks related to nicotine exposure would not be expected to be increased on switching from CC smoking to ENDS use.

87. In the evaluation of absolute risk from exposure to E(N)NDS emissions, the Committee considered adverse health effects that could be of concern include the potential for sensory irritation, the promotion or augmentation of respiratory symptoms in people with respiratory disease or conditions, and the potential to enhance adverse cardiovascular symptoms in people with cardiovascular disease. There is also the risk that nicotine addiction may develop in nicotine-naïve users.

There is good evidence from animal studies for an increased risk of adverse effects on development associated with nicotine exposure, but the data set on this aspect was not adequate for the level of such risk in humans from exposure to ENDS emissions to be assessed. The possibility of adverse effects of nicotine in bystanders cannot be excluded, although in most exposure scenarios the level of exposure to nicotine from ambient air would be low.

88. A relatively large evidence-base of studies was included in the COT review, but many of these data had limitations. Experimental studies with E(N)NDS aerosols are difficult to compare due to the variability in E(N)NDS devices and e-liquid products tested, puffing parameters, experimental set-ups, analytical techniques, and methods for data analysis and reporting. There is a need for standardised and validated testing protocols and for good reporting of studies. Other factors that will, to some extent, limit the applicability of the findings of the COT review are the rapid pace of development in both the E(N)NDS product market and publications of new studies of relevance to potential E(N)NDS-related health effects. The Committee acknowledges that some *in vitro* studies may provide additional useful information e.g. potential for immunotoxicity. A literature update conducted towards the end of the COT review indicated a substantial database of new literature, which appeared, overall, to add weight to the previous findings. The field of studies on potential toxicological effects of E(N)NDS is an area that the COT will keep under review in the future.

89. There have been a number of recent case reports in the US of lung injuries related to the use of E(N)NDS products. These have been investigated by US authorities who have confirmed vitamin E acetate, a thickening agent added to cannabis vaping products, was strongly linked to the US outbreak[19]. This substance is banned from UK-regulated nicotine vaping products. This area sits outside the COT review scope of intended use and falls outside the timescale of the present COT review. However, it is a topic that the Committee will keep a watching brief on as necessary.

90. The COT review identified several important gaps in knowledge on potential toxicological effects of E(N)NDS. There is a large data gap regarding long-term toxicity from E(N)NDS use. It is not known what the nature of the effects would be as they may not be the same as the known effects of CC smoking, and long-term effects of E(N)NDS would become apparent only after many years. Prospective epidemiological studies would help to address this data gap. Other data gaps include the lack of knowledge regarding inhalation exposure to flavouring chemicals, including potential breakdown products, and long-term toxicological effects of inhaled nicotine itself in humans.

91. To ensure toxicological risks are kept to a minimum, the Committee emphasises the need for good production standards for E(N)NDS products. In terms of E(N)NDS devices, this would include aspects such as the fidelity of construction,

[19] https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html

materials used, and operating capabilities. For e-liquids, the formulants should be derived from a reputable source, and non-standard constituents should not be included. Products should be used according to standard operating conditions and should not be modified for atypical use. In addition, there are reports of poisonings occurring[20], both accidental and deliberate, and due consideration should be given to ensuring appropriate storage and to avoid user confusion, e.g. with eye/ear drops.

Overall conclusion

92. The use of E(N)NDS products, produced according to appropriate manufacturing standards and used as recommended, as a replacement for CC smoking, is likely to be associated with a reduction in overall risk of adverse health effects, although the magnitude of the decrease will depend on the effect in question. Uptake of E(N)NDS product use *de novo* by non-users of tobacco products is likely to be associated with some adverse health effects to which the user would not otherwise have been subject. The use of a wide range of flavouring products in e-liquids, for which data on toxicity by inhalation, particularly of any thermally-derived products, are generally not available, is an area of uncertainty. While there is currently no information that this is leading to adverse effects on human health, this is an important data gap. E(N)NDS use is associated with some emissions into ambient air, including nicotine. For most health effects, the risks to bystanders will probably be low in conventional exposure scenarios, although pharmacological effects from exposure to nicotine in ambient air may occur in some individuals.

93. There are large evidence gaps within the literature and available information. It is not possible to fully assess the risks related to all possible constituents in E(N)NDS products. There are very little data available for products that do not contain nicotine (ENNDS). It is not currently possible to predict the adverse health effects that could be associated with use of E(N)NDS products in the long term. This is reflected in the different policies on E(N)NDS across different countries. Information and science relating to E(N)NDS is changing rapidly and the COT will keep this area under review.

COT
July 2020; Statement Number 2020/04

Acknowledgements

The Committee is very grateful to Dr. Tharacad Ramanarayanan and Dr. Doug Wolf, Syngenta, North Carolina, for providing expertise in the risk assessments of propylene glycol and glycerol.

[20] http://www.npis.org/NPISAnnualReport2018-19.pdf

Abbreviations

AQG	Air quality guideline
AR	Absolute risk
CC	Conventional cigarette
COC	Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment
COM	Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment
COPD	Chronic obstructive pulmonary disease
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DHSC	Department of Health and Social Care
EEG	Electro-encephalogram
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
HBGV	Health-based guidance value
HEC	Human equivalent concentration
HR	Heart rate
i.v.	Intra-venous
LOAEC	Lowest observed adverse effect concentration
LOD	Limit of detection
LOQ	Limit of quantitation
MHRA	Medicines and Healthcare products Regulatory Agency
MOS	Margin of safety
NAB	*N*-nitrosoanabasine
NAT	*N*'-nitrosoanatabine
NHS	National Health Service (UK)
NNK	Nicotine-derived nitrosamine ketone
NNN	*N*-nitrosonornicotine
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NRT	Nicotine replacement therapy
PAH	Polycyclic aromatic hydrocarbon
PG	Propylene glycol
PHE	Public Health England
PM	Particulate matter
$PM_{2.5}$	Particulate matter 2.5 μm or less in diameter
PM_{10}	Particulate matter 10 μm or less in diameter
POD	Point of departure
RR	Relative risk
TPD	Tobacco Products Directive
TPM	Total particulate matter
TRP	Transient receptor potential
TSNA	Tobacco-specific nitrosamine
UF	Uncertainty factor

VG	Vegetable glycerin(e)
VOC	Volatile organic compound
WHO	World Health Organization

References

Benowitz, N. L., J. Hukkanen & P. Jacob, 3rd (2009) Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb Exp Pharmacol*, 29-60.

Benowitz, N. L. & P. Jacob, 3rd (1984) Daily intake of nicotine during cigarette smoking. *Clin Pharmacol Ther,* 35, 499-504.

Brady, B. R., J. S. De La Rosa, U. S. Nair & S. J. Leischow (2019) Electronic Cigarette Policy Recommendations: A Scoping Review. *Am J Health Behav,* 43, 88-104.

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*.

Dawkins, L., S. Cox, M. Goniewicz, H. McRobbie, C. Kimber, M. Doig & L. Kosmider (2018) 'Real-world' compensatory behaviour with low nicotine concentration e-liquid: subjective effects and nicotine, acrolein and formaldehyde exposure. *Addiction,* 113, 1874-1882.

ECHA (2017). Guidance on the Biocidal Products Regulation: Volume III Human Health - Assessment & Evaluation (Parts B+C), Version 4.0. ECHA, Helsinki, Finland. https://echa.europa.eu/documents/10162/23036412/biocides_guidance_human_health_ra_iii_part_bc_en.pdf/30d53d7d-9723-7db4-357a-ca68739f5094

Elmore, S., A. Dodson & D. Mottram. 2014. Reactions of Propylene Glycol with the Constituents of Food Flavorings. Proceedings from XIII Weurman Flavour Research Symposium 2014, Flavour science, Pages 577-581.

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

Goniewicz, M. L., J. Knysak, M. Gawron, L. Kosmider, A. Sobczak, J. Kurek, A. Prokopowicz, M. Jablonska-Czapla, C. Rosik-Dulewska, C. Havel, P. Jacob Iii & N. Benowitz (2014) Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tobacco control,* 23, 133-139.

Hartmann-Boyce, J., H. McRobbie, C. Bullen, R. Begh, L. F. Stead & P. Hajek (2016) Electronic cigarettes for smoking cessation. *The Cochrane database of systematic reviews,* 9, CD010216.

HCN (2007) Propylene glycol (1,2-Propanediol); Health-based recommended occupational exposure limit. The Hague:: Health Council of the Netherlands.

Hukkanen, J., P. Jacob, 3rd & N. L. Benowitz (2005) Metabolism and disposition kinetics of nicotine. *Pharmacol Rev,* 57**,** 79-115.

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*.

Kienhuis, A. S., L. G. Soeteman-Hernandez, P. M. J. Bos, H. W. J. M. Cremers, W. N. Klerx & R. Talhout (2015) Potential harmful health effects of inhaling nicotine-free shisha-pen vapor: a chemical risk assessment of the main components propylene glycol and glycerol. *Tobacco Induced Diseases,* 13**,** 15-15.

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

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.

Margham, J., K. McAdam, M. Forster, C. Liu, C. Wright, D. Mariner & C. Proctor (2016) Chemical Composition of Aerosol from an E-Cigarette: A Quantitative Comparison with Cigarette Smoke. *Chem Res Toxicol,* 29**,** 1662-1678.

Mayer, B. (2014) How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century. *Arch Toxicol,* 88**,** 5-7.

Murray, R. P., J. E. Connett & L. M. Zapawa (2009) Does nicotine replacement therapy cause cancer? Evidence from the Lung Health Study. *Nicotine and Tobacco Research,* 11**,** 1076-1082.

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

Renne, R. A., Wehner, A.P., Greenspan, B.J., Deford, H.S., Ragan, H.A., Westerberg, R.B., Buschbom, R.L., Burger, G.T., Hayes, A.W., Suber, R.L., Mosberg, A.T. (1992) 2-week and 13-week inhalation studies of aerosolized glycerol in rats. *Inhalation Toxicology,* 4**,** 95-111.

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.

Sleiman, M., J. M. Logue, V. N. Montesinos, M. L. Russell, M. I. Litter, L. A. Gundel & H. Destaillats (2016) Emissions from Electronic Cigarettes: Key Parameters Affecting the Release of Harmful Chemicals. *Environ Sci Technol,* 50**,** 9644-51.

Suber, R. L., R. Deskin, I. Nikiforov, X. Fouillet & C. R. Coggins (1989) Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. *Food Chem Toxicol,* 27**,** 573-83.

Tayyarah, R. & G. A. Long (2014) Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air. *Regul Toxicol Pharmacol,* 70**,** 704-10.

WHO (2006) WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Global update 2005. Summary of risk assessment.

WHO (2017). Study Group on Tobacco Product Regulation (TobReg) Report on the scientific basis of tobacco product regulation: Sixth report of a WHO study group, TRS 1001. WHO, Geneva, Switzerland. Available at https://apps.who.int/iris/bitstream/handle/10665/260245/9789241210010-eng.pdf?sequence=1

Williams, M., K. Bozhilov, S. Ghai & P. Talbot (2017) Elements including metals in the atomizer and aerosol of disposable electronic cigarettes and electronic hookahs. *PLoS ONE,* 12.

Williams, M., A. Villarreal, K. Bozhilov, S. Lin & P. Talbot (2013) Metal and Silicate Particles Including Nanoparticles Are Present in Electronic Cigarette Cartomizer Fluid and Aerosol. *PLoS ONE,* 8.

Annex A

Discussion papers presented to the COT on the potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS, e-cigarettes).

TOX/2017/49 (13/12/2017)	Paper 1: Characterisation of the aerosol droplet particle fraction
TOX/2018/15 & Annex A (20/03/2018)	Paper 2: Exposure to metals present in the aerosol of electronic nicotine (or non-nicotine) delivery systems
TOX/2018/16 (20/03/2018)	Preparation for further discussion papers
TOX/2018/19 (08/05/2018)	Paper 3: Toxicological review of the main constituents, propylene glycol (PG) and vegetable glycerine (VG, glycerol)
TOX/2018/20 (08/05/2018)	Follow up to paper 2: Additional information on reports describing the presence of silicon/silicates in the aerosol of E(N)NDS
TOX/2018/23 (03/07/2018)	Follow up to paper 3: additional information on 13-week inhalation studies in rats of propylene glycol aerosol (Suber et al., 1989) and glycerol aerosol (Renne et al., 1992)
TOX/2018/24 (03/07/2018)	Paper 4: Toxicological and epidemiological evaluations of E(N)NDS aerosol exposures
TOX/2018/25 (03/07/2018)	Paper 5: Preliminary overview of nicotine toxicity
TOX/2018/45 (04/12/2018)	Paper 6: A review of data relating to developmental toxicity in offspring following parental exposure to nicotine
TOX/2018/46 (04/12/2018)	Paper 7: Additional information on developmental toxicity studies of E(N)NDS aerosols
TOX/2018/47 (04/12/2018)	Paper for information on COM and COC consideration of genotoxicity and carcinogenicity risks
TOX/2018/52 (04/12/2018)	Recent paper hypothesising role of nicotine in schizophrenia spectrum disorders
TOX/2019/01 (06/02/2019)	Paper 8: Additional information on toxicity in adolescent and young adult users

TOX/2019/11 (19/03/2019)	Paper 9: Bystander exposure
TOX/2019/24 (07/05/2019)	Paper 10a: Toxicity assessment of flavourings used in E(N)NDS: Vanillin
TOX/2019/25 (07/05/2019)	Paper 10b: Toxicity assessment of flavourings used in E(N)NDS: Cinnamaldehyde
TOX/2019/37 (02/07/2019)	Paper 11: Decision tree for risk assessing flavouring compounds in E(N)NDS
TOX/2019/38 (02/07/2019)	Paper 12: Toxicological review of nicotine
TOX/2019/39 (02/07/2019)	Paper 13: User exposure
TOX/2019/46 (17/09/2019)	Follow up to Paper 12: An overview of strategies to reduce nicotine addiction using low-nicotine-content products
TOX/2019/47 (17/09/2019)	Follow up to Paper 12: Calculation of a health-based guidance value for inhalation exposure to nicotine based on the study of Lindgren et al. (1999)
TOX/2019/48 (17/09/2019)	Paper 10c: Toxicity assessment of flavourings used in E(N)NDS: Menthol
TOX/2019/49 (17/09/2019)	Follow up to paper 11: Second draft framework for risk assessment of flavouring compounds in E(N)NDS
TOX/2019/50 (17/09/2019)	Literature update to mid-2019
TOX/2019/58 (22/10/2019)	Paper 10d: Toxicity assessment of flavourings used in E(N)NDS: Menthone
TOX/2019/59 (22/10/2019)	Follow-up to Paper 13: Tabulation of user exposure data
TOX/2019/60 (22/10/2019)	Follow-up to Literature update to mid-2019 – further details of publications in TOX/2019/50
TOX/2019/72 (03/12/2019)	Follow-up from September 2019 COT meeting: updated risk assessments for nicotine exposure from ENDS
TOX/2020/05 (28/01/2020)	Reviews by UK and International organisations

TOX/2020/06 (28/01/2020)	Paper for Information: Relevant UK regulatory aspects
TOX/2020/07 (28/01/2020)	Paper for Information: Data from MHRA Yellow Card Scheme
TOX/2020/29 & Annex A (05/05/2020)	Updated risk assessments for exposure of users to propylene glycol (PG) and glycerol from inhalation of E(N)NDS aerosols

Framework for risk assessment of flavouring compounds

The framework for risk assessment of flavouring compounds (COT Statement 2020/01) provides a number of steps designed as a set of principles to guide the risk assessment process for a flavouring compound in E(N)NDS. It assumes some level of expertise of the assessor. Existing data or non-animal approaches should be used to inform each step where possible. The steps are illustrated in Figure 1, below.

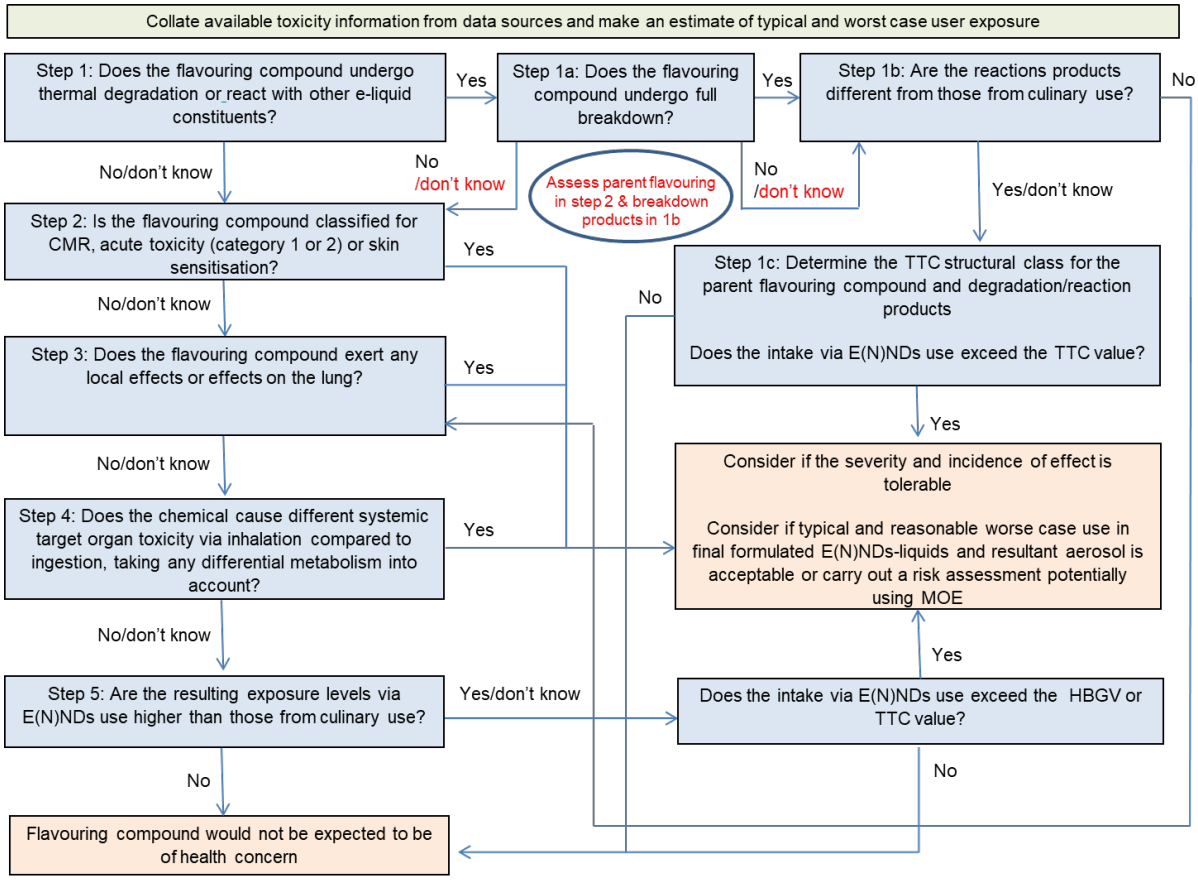

Figure 1. Framework for risk assessment of flavouring compounds via inhalation exposure (from COT Statement 2020/01)

CASE STUDY: VANILLIN

This is an illustrative case study on use of the framework; the Committee has previously considered vanillin in paper TOX/2019/24.

STEP 1 Does the flavouring compound undergo thermal degradation or react with other e-liquid constituents?

1. Aldehydes and alcohols can undergo chemical reactions to form aldehyde PG acetal. Therefore, Erythropel et al. (2018) hypothesised that vanillin could react with PG and VG, commonly found in E(N)NDs liquids, to form vanillin propylene glycol acetal.

STEP 1a Does the flavouring compound undergo full breakdown?

2. Experiments demonstrated that vanillin rapidly reacted with PG after mixing, and <40% was converted to vanillin propylene glycol acetal. This was measured in E(N)NDs liquids and E(N)NDs vapour. Costigan et al. (2014) also reported that vanillin propylene glycol acetal was present in e-cigarette aerosol of an experimental flavoured formulation that was not present in the parent flavour.

3. The analytical studies did not report the concentrations of the flavour aldehyde acetals in the respective e-liquids, and it remains unclear how frequently and how rapidly these compounds form and whether they remain stable during heating and vaporization in e-cigarettes (Erythropel et al., 2018).

STEP 1b Are the reaction products different from those from culinary use?

4. Elmore *et al.* (2014) reported that under acidic or basic conditions, PG can react with food flavourings to give rise to new compounds. However, no data specific to vanillin could be found. For the purposes of this illustration, a ‘don’t know’ answer is assumed. However, when using the framework for flavouring compounds this information must be sought.

STEP 1c Determine the TTC structural class for the flavouring compound and degradation/reaction products. Does the intake via E(N)NDs use exceed the TTC value?

5. Vanillin and vanillin propylene glycol acetal are categorised as TTC class I (low toxicity) and III (high toxicity), respectively.

6. Exposure to vanillin and vanillin propylene glycol acetal via E(N)NDs use would need to be calculated using generic assumptions.

Outcome of step 1

7. Vanillin undergoes degradation to form vanillin propylene glycol acetal. However, it is unknown whether such reactions are similar to culinary use or specific to E(N)NDs use hence a TTC approach for the flavouring compound and degradation product should be used.

8. If the intake is lower than the TTC value then the flavouring compound would not be expected to be of health concern. If higher than the TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

STEP 2 Is the flavouring compound classified for CMR, acute toxicity (category 1 or 2) or skin sensitisation?

Carcinogenicity / Mutagenicity / Reproductive and developmental toxicity

IARC: No evaluation

Harmonised classification for CMR: Not available

Candidate list of substances of very high concern (SVHCs): Not on SVHC list

QSARs – ToxTree

Carcinogenicity (genotox and nongenotox) and mutagenicity rulebase by ISS: Structural alert for genotoxic carcinogenicity (simple aldehyde); negative for nongenotoxic carcinogenicity

In vitro mutagenicity (Ames) alerts by ISS: Structural alert for S.typhimurium mutagenicity (simple aldehyde)

Structural alerts for the in vivo micronucleus assay in rodents: Micronucleus assay; at least one positive structural alert

DNA binding alert: Alert for Michael Acceptor identified

QSARs – VEGA

Mutagenicity (Ames) test model: Non-mutagenic

Carcinogenicity model: Carcinogen/possible non-carcinogen

Carcinogenicity inhalation classification model: Non-carcinogen but results may be unreliable

Self-notified C+L classification: Not classified for CMR

Acute toxicity

Harmonised classification: Not available

Self-notified C+L classification: Not classified for acute toxicity (inhalation)

Skin Sensitisation

Harmonised classification: Not available

QSARs – Toxtree

> Skin sensitisation reactivity domains: Alert for Michael Acceptor identified; alert for Schiff base formation identified
>
> Protein binding alerts: Alert for Michael Acceptor identified; alert for Schiff base formation identified

QSARs – VEGA

> Skin sensitisation model: Non sensitiser

Self-notified C+L classification: Classified for skin sensitisation category 1 (H317; may cause an allergic skin reaction) in 7/25 aggregated notifications

Clinical reports and observations: Animal and human data indicate it is not a skin sensitiser

Outcome of step 2

9. Vanillin does not have a harmonised classification under classification, labelling and packaging (CLP) and is not classified for CMR or acute toxicity via inhalation. It does have a self-notified classification for skin sensitisation under CLP (self-notifications).

10. Equivocal data for mutagenicity and carcinogenicity were obtained from QSAR models used (ToxTree and VEGA), whereas both models showed vanillin not to be a skin sensitiser.

11. Based on a weight of evidence approach, using the classification and labelling (C+L) notifications and the QSAR predictions, vanillin is not considered CMR, an acute toxin via inhalation or skin sensitiser.

STEP 3 Does the flavouring compound exert any local effects or effects on the lung?

Respiratory irritation

Harmonised classification: Not available

Self-notified C+L classification: Classified as STOT SE category 3 (lungs/inhalation) (H335; may cause respiratory irritation) in 1/25 aggregated notifications

RD_{50} (*in vivo* data/*in vitro* data/physchem data): Calculated RD_{50} = 2.00 ppm

Clinical reports and observations: Activates TRPA1 receptors – may act as a sensory irritant

Respiratory Sensitisation

Harmonised classification: Not available

Candidate list of SVHCs: Not on SVHC list for respiratory sensitisation

Self-notified C+L classification: Not classified for respiratory sensitisation

Clinical reports and observations: No data on respiratory sensitisation available

Effect on the lung

Harmonised classification: Not available

Candidate list of SVHCs: Not on SVHC list

Self-notified C+L classification: Not classified for STOT RE

Clinical reports and observations: No data available

Outcome of step 3

12. Vanillin is classified as STOT SE category 3 (may cause respiratory irritation), noting lungs and the respiratory system as the target organ but is not classified as STOT RE. It activates the TRP receptors indicative of vanillin being a sensory irritant. There are insufficient data to evaluate the respiratory sensitisation potential.

13. The use of vanillin in e-liquids may be undesirable based on the potential to cause respiratory irritation through activation of TRP receptors. The severity and incidence of the effect should be considered to see if it is tolerable. Typical and reasonable worse case use in final formulated E(N)NDs liquids and aerosols should be considered to see if they are acceptable or risk assessment, potentially using MOE, should be carried out.

STEP 4 Does the chemical cause different systemic target organ toxicity via inhalation compared to ingestion, taking any differential metabolism into account?

14. Few data are available on the toxicity of vanillin via inhalation.

STEP 5 Are the resulting exposure levels via E(N)NDs use higher than those from culinary use?

15. Exposure to vanillin via E(N)NDs use would need to be calculated using generic assumptions (see step 1c).

Outcome of step 5

16. It is unknown whether exposure to vanillin via E(N)NDs use is similar or lower than culinary use. Hence intake should be compared to a HBGV or the TTC value.

17. If the intake is lower than the HBGV or TTC value then the flavouring compound would not be expected to be of health concern. If higher than the HBGV or TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

Summary

18. The risk assessment framework was followed for vanillin. It undergoes partial reaction with PG to form vanillin propylene glycol acetal. Therefore, both vanillin propylene glycol acetal and vanillin should be assessed using the framework. Moreover, it is uncertain whether this reaction is specific to E(N)NDs or also occurs in culinary use hence the TTC approach for both compounds should be carried out.

19. Vanillin was not classified as CMR, with high acute toxicity or as a skin sensitiser in step 2.

20. In step 3, vanillin was classified as STOT SE based on respiratory irritation hence its use in e-liquids may be undesirable. Typical and reasonable worse case use in final formulated E(N)NDs liquids and aerosols should be considered to see if they are acceptable or risk assessment, potentially using MOE, should be carried out.

21. A comparison of systemic target organ toxicity following inhalation or ingestion could not be carried out in step 4 due to the lack of data following inhalation.

22. In step 5, as in step 1c, an exposure assessment via E(N)NDs use should be carried out and levels compared with a HBGV or TTC values. If the intake is lower than the TTC value then the flavouring compound would not be expected to be of health concern. If higher than the TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

References

Carthew, P., Clapp, C. and Gutsell, S. (2009) Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for

aerosol ingredients in consumer products. . Food and Chemical Toxicology, 47, 1287-1295.

Costigan, S., Lang, B. and Collard, J. (2014) Risk assessment approach for E-cigarette flavours. EUROTOX poster.

Czégény, Z., Bozi, J., Sebestyén, Z., Blazsó, M., Jakab, E., Barta-Rajnai, E., Forster, M., Nicol, J., McAdam, K.G. and Liu, C. (2016) Thermal behaviour of selected flavour ingredients and additives under simulated cigarette combustion and tobacco heating conditions. Journal of Analytical and Applied Pyrolysis, 121, 190-204.

ECHA (2017) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance. ECHA-17-G-18-EN.

EFSA (2012) Scientific Opinion on Flavouring Group Evaluation 9, Revision 4 (FGE.09Rev4): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25. EFSA journal, 10 (7).

EFSA/WHO (2016) Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree. EFSA supporting publication 2016: EN-1006. 50 pp.

Elmore, S., Dodson, A. and Mottram, D. (2014) Reactions of Propylene Glycol with the Constituents of Food Flavorings. Proceedings from XIII Weurman Flavour Research Symposium 2014, Flavour science, Pages 577-581.

Escher, S., Tluczkiewicz, I., Batke, M., Bitsch, A., Melber, C., Kroese, E., Buist, H. and Mangelsdorf, I. (2010) Evaluation of inhalation TTC values with the database RepDose. . Regul. Toxicol. Pharmacol. , 58, 259-274.

GHS (2017) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Seventh revised edition United Nations.

JECFA (1999) Substances structurally related to menthol. International programme on chemical safety, WHO FOOD ADDITIVES SERIES: 42.

Kuwabara, Y., Alexeeff, G.V., Broadwin R. and Salmon, A.G. (2007) Evaluation and Application of the RD50 for Determining Acceptable Exposure Levels of Airborne Sensory Irritants for the General Public. Environmental Health Perspectives, 115, 1609-1616.

SCHEER (2016) Additives used in tobacco products, Opinion II, 16 December 2016.

CASE STUDY: CINNAMALDEHYDE

This is an illustrative case study on use of the framework; the Committee has previously considered cinnamaldehyde in paper TOX/2019/25.

STEP 1 Does the flavouring compound undergo thermal degradation or react with other e-liquid constituents?

1. Aldehydes and alcohols can undergo chemical reactions to form aldehyde PG acetal. Therefore, Erythropel et al. (2018) hypothesised that cinnamaldehyde could react with PG and VG, commonly found in E(N)NDs liquids, to form cinnamaldehyde propylene glycol acetal.

STEP 1a Does the flavouring compound undergo full breakdown?

2. Experiments demonstrated that cinnamaldehyde rapidly reacted with PG after mixing, and <40% was converted to cinnamaldehyde propylene glycol acetal. This was measured in E(N)NDs liquids and E(N)NDs vapour. Costigan et al. (2014) also reported that cinnamaldehyde propylene glycol acetal was present in e-cigarette aerosol of an experimental flavoured formulation that was not present in the parent flavour.

3. The analytical studies did not report the concentrations of the flavour aldehyde acetals in the respective e-liquids, and it remains unclear how frequently and how rapidly these compounds form and whether they remain stable during heating and vaporization in e-cigarettes (Erythropel et al., 2018).

STEP 1b Are the reaction products different from those from culinary use?

4. Elmore *et al.* (2014) reported that under acidic or basic conditions, PG can react with food flavourings to give rise to new compounds. However, no data specific to cinnamaldehyde could be found. For the purposes of this illustration, a ‘don’t know’ answer is assumed. However, when using the framework for flavouring compounds this information must be sought.

STEP 1c Determine the TTC structural class for the flavouring compound and degradation/reaction products. Does the intake via E(N)NDs use exceed the TTC value?

5. Cinnamaldehyde and cinnamaldehyde propylene glycol acetal are categorised as TTC class I (low toxicity) and III (high toxicity), respectively.

6. Exposure to cinnamaldehyde and cinnamaldehyde propylene glycol acetal via E(N)NDs use would need to be calculated using generic assumptions.

Outcome of step 1

7. Cinnamaldehyde undergoes degradation to form cinnamaldehyde propylene glycol acetal. However, it is unknown whether such reactions are similar to culinary use or specific to E(N)NDs use hence a TTC approach for the flavouring compound and degradation product should be used.

8. If the intake is lower than the TTC value then the flavouring compound would not be expected to be of health concern. If higher than the TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

STEP 2 Is the flavouring compound classified for CMR, acute toxicity (category 1 or 2) or skin sensitisation?

Carcinogenicity / Mutagenicity / Reproductive and developmental toxicity

IARC: Not carcinogenic

Harmonised classification for CMR: Not available

Candidate list of substances of very high concern (SVHCs): Not on SVHC list

QSARs – ToxTree

Carcinogenicity (genotox and nongenotox) and mutagenicity rulebase by ISS: Negative for genotoxic and nongenotoxic carcinogenicity; potential S-typhimurium TA100 mutagen (α,β unsaturated aliphatic aldehyde)

In vitro mutagenicity (Ames) alerts by ISS: No alerts; potential S-typhimurium TA100 mutagen (α,β unsaturated aliphatic aldehyde)

Structural alerts for the in vivo micronucleus assay in rodents: No alerts

DNA binding alert: Alert for Michael Acceptor identified

QSARs – VEGA

Mutagenicity (Ames) test model: Non-mutagenic

Carcinogenicity model: Non-carcinogen

Carcinogenicity inhalation classification model: Non-carcinogen

Self-notified C+L classification: Not classified for CMR

Acute toxicity

Harmonised classification: Not available

Self-notified C+L classification: Not classified for acute toxicity (inhalation)

Skin Sensitisation

Harmonised classification: Not available

QSARs – Toxtree

Skin sensitisation reactivity domains: Alert for Michael Acceptor identified

Protein binding alerts: Alert for Michael Acceptor identified

QSARs – VEGA

Skin sensitisation model: Sensitiser

Self-notified C+L classification: Classified for skin sensitisation category 1 (H317; may cause an allergic skin reaction) in 24/31 aggregated notifications

Clinical reports and observations: Animal and human data indicate it is a skin sensitiser

Outcome of step 2

9. Cinnamaldehyde does not have a harmonised classification under classification, labelling and packaging (CLP) and is not classified for CMR or acute toxicity via inhalation. It does have a self-notified classification for skin sensitisation under CLP (self-notifications).

10. No alerts for mutagenicity or carcinogenicity were obtained from QSAR models used (ToxTree and VEGA), whereas both models showed cinnamaldehyde to be a skin sensitiser.

11. Based on a weight of evidence approach, using the classification and labelling (C+L) notifications and the QSAR predictions, cinnamaldehyde is not considered CMR or an acute toxin via inhalation but it is a skin sensitiser.

12. The use of cinnamaldehyde in e-liquids may be undesirable based on the potential to cause skin sensitisation. The severity and incidence of the effect should be considered to see if it is tolerable. Typical and reasonable worse case use in final formulated E(N)NDs liquids and aerosols should be considered to see if they are acceptable or risk assessment, potentially using MOE, should be carried out.

STEP 3 Does the flavouring compound exert any local effects or effects on the lung?

Respiratory irritation

Harmonised classification: Not available

Self-notified C+L classification: Classified as STOT SE category 3 (lungs) (H335; may cause respiratory irritation) in 4/31 aggregated notifications

RD_{50} (*in vivo* data/*in vitro* data/physchem data): Calculated RD_{50} = 68 ppm

Clinical reports and observations: Activates TRPA1 receptors – may act as a sensory irritant

Respiratory Sensitisation

Harmonised classification: Not available

Candidate list of SVHCs: Not on SVHC list for respiratory sensitisation

Self-notified C+L classification: Not classified for respiratory sensitisation

Clinical reports and observations: No data on respiratory sensitisation available

Effect on the lung

Harmonised classification: Not available

Candidate list of SVHCs: Not on SVHC list

Self-notified C+L classification: Not classified for STOT RE

Clinical reports and observations: No data available

Outcome of step 3

13. Cinnamaldehyde is classified as STOT SE category 3 (may cause respiratory irritation), noting lungs and the respiratory system as the target organ but is not classified as STOT RE. It activates the TRP receptors indicative of cinnamaldehyde being a sensory irritant. There are insufficient data to evaluate the respiratory sensitisation potential.

14. The use of cinnamaldehyde in e-liquids may be undesirable based on the potential to cause respiratory irritation through activation of TRP receptors. The severity and incidence of the effect should be considered to see if it is tolerable. Typical and reasonable worse case use in final formulated E(N)NDs liquids and aerosols should be considered to see if they are acceptable or risk assessment, potentially using MOE, should be carried out.

STEP 4 Does the chemical cause different systemic target organ toxicity via inhalation compared to ingestion, taking any differential metabolism into account?

15. Few data are available on the toxicity of cinnamaldehyde via inhalation.

STEP 5 Are the resulting exposure levels via E(N)NDs use higher than those from culinary use?

16. Exposure to cinnamaldehyde via E(N)NDs use would need to be calculated using generic assumptions (see step 1c).

Outcome of step 5

17. It is unknown whether exposure to cinnamaldehyde via E(N)NDs use is similar or lower than culinary use. Hence intake should be compared to a HBGV or the TTC value.

18. If the intake is lower than the HBGV or TTC value then the flavouring compound would not be expected to be of health concern. If higher than the HBGV or TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

Summary

19. The risk assessment framework was followed for cinnamaldehyde. It undergoes partial reaction with PG to form cinnamaldehyde propylene glycol acetal. Therefore, both cinnamaldehyde propylene glycol acetal and cinnamaldehyde should be assessed using the framework. Moreover, it is uncertain whether this reaction is specific to E(N)NDs or also occurs in culinary use hence the TTC approach for both compounds should be carried out.

20. Cinnamaldehyde was not classified as CMR or high acute toxicity. It is classified as a skin sensitiser in step 2, and as STOT SE in step 3, hence its use in e-liquids may be undesirable. Typical and reasonable worse case use in final formulated E(N)NDs liquids and aerosols should be considered to see if they are acceptable or risk assessment, potentially using MOE, should be carried out.

21. A comparison of systemic target organ toxicity following inhalation or ingestion could not be carried out in step 4 due to the lack of data following inhalation.

22. In step 5, as in step 1c, an exposure assessment via E(N)NDs use should be carried out and levels compared with a HBGV or TTC values. If the intake is lower than the TTC value then the flavouring compound would not be expected to be of

health concern. If higher than the TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

References

Carthew, P., Clapp, C. and Gutsell, S. (2009) Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. . Food and Chemical Toxicology, 47, 1287-1295.

Costigan, S., Lang, B. and Collard, J. (2014) Risk assessment approach for E-cigarette flavours. EUROTOX poster.

Czégény, Z., Bozi, J., Sebestyén, Z., Blazsó, M., Jakab, E., Barta-Rajnai, E., Forster, M., Nicol, J., McAdam, K.G. and Liu, C. (2016) Thermal behaviour of selected flavour ingredients and additives under simulated cigarette combustion and tobacco heating conditions. Journal of Analytical and Applied Pyrolysis, 121, 190-204.

ECHA (2017) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance. ECHA-17-G-18-EN.

EFSA (2012) Scientific Opinion on Flavouring Group Evaluation 9, Revision 4 (FGE.09Rev4): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25. EFSA journal, 10 (7).

EFSA/WHO (2016) Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree. EFSA supporting publication 2016: EN-1006. 50 pp.

Elmore, S., Dodson, A. and Mottram, D. (2014) Reactions of Propylene Glycol with the Constituents of Food Flavorings. Proceedings from XIII Weurman Flavour Research Symposium 2014, Flavour science, Pages 577-581.

Escher, S., Tluczkiewicz, I., Batke, M., Bitsch, A., Melber, C., Kroese, E., Buist, H. and Mangelsdorf, I. (2010) Evaluation of inhalation TTC values with the database RepDose. . Regul. Toxicol. Pharmacol. , 58, 259-274.

GHS (2017) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Seventh revised edition United Nations.

JECFA (1999) Substances structurally related to menthol. International programme on chemical safety, WHO FOOD ADDITIVES SERIES: 42.

Kuwabara, Y., Alexeeff, G.V., Broadwin R. and Salmon, A.G. (2007) Evaluation and Application of the RD50 for Determining Acceptable Exposure Levels of Airborne Sensory Irritants for the General Public. Environmental Health Perspectives, 115, 1609-1616.

SCHEER (2016) Additives used in tobacco products, Opinion II, 16 December 2016.

CASE STUDY: MENTHOL

This is an illustrative case study on use of the framework; the Committee will consider menthol in paper TOX/2019/48.

STEP 1 Does the flavouring compound undergo thermal degradation or react with other e-liquid constituents?

1. Menthol is reported to be converted to menthone, mentene and menthane upon pyrolysis (SCHEER, 2016). Czégény *et al.* (2016) carried out a study to mimic pyrolysis conditions at low temperature heating. Using a 300 ˚C isothermal temperature for 5 minutes, menthol was converted to menthone and menthene in an oxygen atmosphere, but not in a nitrogen atmosphere. Menthol may also react with propylene glycol forming menthol propylene glycol carbonate, which is also used as a food flavouring (EFSA, 2012).

STEP 1a Does the flavouring compound undergo full breakdown?

2. After pyrolysis of menthol, it is transferred intact into smoke (99%) (Baker and Bishop, 2004; Jenkins, 1970 cited in SCHEER, 2016). Smoking studies resulted in intact transfer of around 98-99% with some formation of menthone, menthene and menthane (SCHEER, 2016). In contrast, in earlier pyrolysis experiments, 84% of the menthol was pyrolysed and phenol and benzo[a]pyrene were found in the pyrolysate (Schmeltz and Schlotzhauer, 1968 cited in SCHEER, 2016).

STEP 1b Are the reaction products different from those from culinary use?

3. Elmore *et al.* (2014) reported that under acidic or basic conditions, PG can react with food flavourings to give rise to new compounds. However, no data specific to menthol could be found. For the purposes of this illustration, a ‘don’t know’ answer is assumed. However, when using the framework for flavouring compounds this information must be sought.

STEP 1c Determine the TTC structural class for the flavouring compound and degradation/reaction products. Does the intake via E(N)NDs use exceed the TTC value?

4. Menthol, menthene and menthane are categorised as TTC class I (low toxicity), and menthone as II (intermediate toxicity).

5. Exposure to menthol, menthone, menthene and menthane via E(N)NDs use would need to be calculated using generic assumptions.

Outcome of step 1

6. Menthol undergoes degradation to form menthone, menthene and menthane. However, it is unknown whether such reactions are similar to culinary use or specific

to E(N)NDs use hence a TTC approach for the flavouring compound and degradation products should be used.

7. If the intake is lower than the TTC value then the flavouring compound would not be expected to be of health concern. If higher than the TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

STEP 2 Is the flavouring compound classified for CMR, acute toxicity (category 1 or 2) or skin sensitisation?

Carcinogenicity / Mutagenicity / Reproductive and developmental toxicity

IARC: Not carcinogenic

Harmonised classification for CMR: Not available

Candidate list of substances of very high concern (SVHCs): Not on SVHC list

QSARs – ToxTree

Carcinogenicity (genotox and nongenotox) and mutagenicity rulebase by ISS: Negative for genotoxic and nongenotoxic carcinogenicity

In vitro mutagenicity (Ames) alerts by ISS: No alerts

Structural alerts for the in vivo micronucleus assay in rodents: No alerts

DNA binding alert: No alerts

QSARs – VEGA

Mutagenicity (Ames) test model: Non-mutagenic

Carcinogenicity model: Non-carcinogen

Carcinogenicity inhalation classification model: Non- carcinogen

Self-notified C+L classification: Not classified for CMR

Acute toxicity

Harmonised classification: Not available

Self-notified C+L classification: Not classified for acute toxicity (inhalation)

Skin Sensitisation

Harmonised classification: Not available

QSARs – Toxtree

Skin sensitisation reactivity domains: No alerts

Protein binding alerts: No alerts

QSARs – VEGA

Skin sensitisation model: Sensitiser/non sensitiser

Self-notified C+L classification: Not classified as a skin sensitiser

Clinical reports and observations: Animal and human data indicate it is not a skin sensitiser

Outcome of step 2

8. Menthol does not have a harmonised classification under classification, labelling and packaging (CLP) and is not classified for CMR, acute toxicity via inhalation or skin sensitisation under CLP (self-notifications).

9. No alerts for mutagenicity, carcinogenicity or skin sensitisation were obtained from QSAR models used (ToxTree and VEGA).

10. Based on a weight of evidence approach, using the classification and labelling (C+L) notifications and the QSAR predictions, menthol is not considered CMR, an acute toxin via inhalation or skin sensitiser.

STEP 3 Does the flavouring compound exert any local effects or effects on the lung?

Respiratory irritation

Harmonised classification: Not available

Self-notified C+L classification: Classified as STOT SE category 3 (lungs) (H335; may cause respiratory irritation) in 2/17 aggregated notifications (menthol), 6/23 (L-menthol); 2/10 (DL-menthol)

RD_{50} (*in vivo* data/*in vitro* data/physchem data): Calculated RD_{50} = 17 ppm (menthol); 27 ppm (L-menthol); 8 ppm (D-menthol)

Clinical reports and observations: Activates TRPM8 receptors – contributes to analgesic and counterirritant properties

Respiratory Sensitisation

Harmonised classification: Not available

Candidate list of SVHCs: Not on SVHC list for respiratory sensitisation

Self-notified C+L classification: Not classified for respiratory sensitisation

Clinical reports and observations: No data on respiratory sensitisation available

Effect on the lung

Harmonised classification: Not available

Candidate list of SVHCs: Not on SVHC list

Self-notified C+L classification: Not classified for STOT RE

Clinical reports and observations: No data available

Outcome of step 3

11. Menthol is classified as STOT SE category 3 (may cause respiratory irritation), noting lungs and the respiratory system as the target organ but is not classified as STOT RE. It activates the TRPM8 receptors indicative of menthol having analgesic and counterirritant properties. There are insufficient data to evaluate the respiratory sensitisation potential.

12. Based on the data available, menthol does not appear to exert adverse local effects or effects on the lung.

STEP 4 Does the chemical cause different systemic target organ toxicity via inhalation compared to ingestion, taking any differential metabolism into account?

13. Following exposure to menthol via inhalation (type of inhalation unknown), mice were reported to have ‘regressive changes’ in the liver and kidney, representing symptoms of the chronic intoxication. No further details are available (Kowalski et al., 1962 cited in ECHA, 2019).

14. Slightly decreased body weights were seen following exposure to menthol in the diet in some studies (ECHA, 2019). Liver weights were significantly increased in gavage studies, although data on the magnitude and incidence are not available (Thorup et al., 1983 cited in ECHA, 2019).

STEP 5 Are the resulting exposure levels via E(N)NDs use higher than those from culinary use?

15. Exposure to menthol via E(N)NDs use would need to be calculated using generic assumptions (see step 1c).

Outcome of step 5

16. It is unknown whether exposure to menthol via E(N)NDs use is similar or lower than culinary use. Hence intake should be compared to a HBGV or the TTC value.

17. If the intake is lower than the HBGV or TTC value then the flavouring compound would not be expected to be of health concern. If higher than the HBGV or TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

Summary

18. The risk assessment framework was followed for menthol. It undergoes degradation to form menthone, menthene and menthane, albeit at low concentrations. Therefore menthol, menthone, menthene and menthane should be assessed using the framework. Moreover, it is uncertain whether this reaction is specific to E(N)NDs or also occurs in culinary use hence the TTC approach for all compounds should be carried out.

19. Menthol was not classified as CMR, with high acute toxicity or as a skin sensitiser in step 2.

20. In step 3, menthol was classified as STOT SE based on respiratory irritation hence its use in e-liquids may be undesirable. Typical and reasonable worse case use in final formulated E(N)NDs liquids and aerosols should be considered to see if they are acceptable or risk assessment, potentially using MOE, should be carried out.

21. A robust comparison of systemic target organ toxicity following inhalation or ingestion could not be carried out in step 4 due to the lack of good quality data.

22. In step 5, as in step 1c, an exposure assessment via E(N)NDs use should be carried out and levels compared with a HBGV or TTC values. If the intake is lower than the TTC value then the flavouring compound would not be expected to be of health concern. If higher than the TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

References

Czégény, Z., Bozi, J., Sebestyén, Z., Blazsó, M., Jakab, E., Barta-Rajnai, E., Forster, M., Nicol, J., McAdam, K.G. and Liu, C. (2016) Thermal behaviour of selected flavour ingredients and additives under simulated cigarette combustion and tobacco heating conditions. Journal of Analytical and Applied Pyrolysis, 121, 190-204.

ECHA (2019) REACH registration dossier. Menthol. . https://echa.europa.eu/registration-dossier/-/registered-dossier/13758/1 Accessed July 2019.

EFSA (2012) Scientific Opinion on Flavouring Group Evaluation 9, Revision 4 (FGE.09Rev4): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25. EFSA journal, 10 (7).

Elmore, S., Dodson, A. and Mottram, D. (2014) Reactions of Propylene Glycol with the Constituents of Food Flavorings. Proceedings from XIII Weurman Flavour Research Symposium 2014, Flavour science, Pages 577-581.

SCHEER (2016) Additives used in tobacco products, Opinion II, 16 December 2016.

CASE STUDY: MENTHONE

This is an illustrative case study for use of the framework; the Committee has previously considered the toxicity profile of menthone in paper (TOX/2019/58).

STEP 1 Does the flavouring compound undergo thermal degradation or react with other e-liquid constituents?

1. No data were found regarding the thermal degradation of menthone or L-menthone or reaction with other constituents of e-liquids.

STEP 1a Does the flavouring compound undergo full breakdown?

2. No data were found regarding the thermal degradation of menthone or L-menthone.

STEP 1b Are the reaction products different from those from culinary use?

3. Elmore, Dodson and Mottram (2014) reported that under acidic or basic conditions, PG can react with food flavourings to give rise to new compounds. However, no data specific to menthone could be found. For the purposes of this illustration, a 'don't know' answer is assumed. However, when using the framework for flavouring compounds information on reaction products must be sought.

STEP 1c Determine the TTC structural class for the flavouring compound and degradation/reaction products. Does the intake via E(N)NDs use exceed the TTC value?

4. Menthone is categorised as threshold of toxicological concern (TTC) class II (intermediate toxicity).

5. Exposure to menthone via E(N)NDs use would need to be calculated using generic assumptions.

Outcome of step 1

6. No data were found regarding the thermal degradation of menthone.

7. If the intake is lower than the TTC value then the flavouring compound would not be expected to be of health concern. If higher than the TTC value, a risk management decision will need to be taken regarding how appropriate its use in E(N)NDs liquids is.

STEP 2 Is the flavouring compound classified for CMR, acute toxicity (category 1 or 2) or skin sensitisation?

Carcinogenicity / Mutagenicity / Reproductive and developmental toxicity

IARC: No data available

Harmonised classification for CMR: Not available

Candidate list of substances of very high concern (SVHCs): Not on SVHC list

QSARs – ToxTree

Carcinogenicity (genotox and nongenotox) and mutagenicity rulebase by ISS: Negative for genotoxic and nongenotoxic carcinogenicity

In vitro mutagenicity (Ames) alerts by ISS: No alerts

Structural alerts for the in vivo micronucleus assay in rodents: No alerts

DNA binding alert: No alerts

QSARs – VEGA

Mutagenicity (Ames) test model: Non-mutagenic

Carcinogenicity model: Carcinogen; may not be reliable

Carcinogenicity inhalation classification model: Non-carcinogen

Self-notified C+L classification: Not classified for CMR

Acute toxicity

Harmonised classification: Not available

Self-notified C+L classification: Classified for acute toxicity category 4; (H302; harmful if swallowed) in 3/12 aggregated notifications

Skin sensitisation

Harmonised classification: Not available

QSARs – Toxtree

Skin sensitisation reactivity domains: No alerts

Protein binding alerts: No alerts

QSARs – VEGA

Skin sensitisation model: Sensitiser

Self-notified C+L classification: Classified for skin sensitisation category 1 (H317; may cause an allergic skin reaction) in 3/12 aggregated notifications

Clinical reports and observations: Animal and human data indicate it is not a skin sensitiser

Outcome of step 2

8. Menthone does not have a harmonised classification under the classification, labelling and packaging (CLP) scheme and is not classified for CMR or acute toxicity via inhalation under CLP (self-notifications). It has a self-notified classification as a category 1 skin sensitisor.

9. No alerts for mutagenicity, or skin sensitisation were obtained from the QSAR models used (ToxTree and VEGA). VEGA gave one alert for carcinogenicity. This may not be reliable as the data for the read across chemicals it was based on were inaccurate.

10. Based on a weight of evidence approach, using the classification and labelling (C+L) notifications according to CLP criteria and the QSAR predictions, menthone is not considered to be a CMR, an acute toxin via inhalation, or a skin sensitiser.

STEP 3 Does the flavouring compound exert any local effects or effects on the lung?

Respiratory irritation

Harmonised classification: Not available

Self-notified C+L classification: Not classified for respiratory irritation

RD_{50} (*in vivo* data/*in vitro* data/physchem data): Calculated RD_{50} = 745 ppm (menthone); 175 ppm (L-menthone)

Clinical reports and observations: No data on respiratory irritation available

Respiratory sensitisation

Harmonised classification: Not available

Candidate list of SVHCs: Not on SVHC list

Self-notified C+L classification: Not classified for respiratory sensitisation

Clinical reports and observations: No data on respiratory sensitisation available

Effect on the lung

Harmonised classification: Not available

Candidate list of SVHCs: Not on SVHC list

Self-notified C+L classification: Not classified for STOT RE

Clinical reports and observations: No data available

Outcome of step 3

11. Menthone is not classified for respiratory effects. Based on the limited data available from animal studies, menthone does not appear to exert adverse local effects or effects on the lung.

STEP 4 Does the chemical cause different systemic target organ toxicity via inhalation compared to ingestion, taking any differential metabolism into account?

12. No data were found regarding the repeated dose toxicity following inhalation exposure to menthone or L-menthone.

STEP 5 Are the resulting exposure levels via E(N)NDs use higher than those from culinary use?

13. Exposure to menthone via E(N)NDs use would need to be calculated using generic assumptions (see step 1c).

Outcome of step 5

14. It is unknown whether exposure to menthone via E(N)NDs use is similar or lower than culinary use. Hence intake should be compared to a health based guidance value (HBGV) or the TTC value.

15. If the intake is lower than the HBGV or TTC value then the flavouring compound would not be expected to be of health concern. If higher than the HBGV or TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

Summary

16. The risk assessment framework was followed for menthone.

17. Menthone was not classified as CMR, as having high acute toxicity or as a skin sensitiser in step 2.

18. In step 3, menthone was not classified for respiratory effects. Based on the limited data it does not exert local effects of effects on the lung.

19. A comparison of systemic target organ toxicity following inhalation or ingestion could not be carried out in step 4 due to the lack of data following inhalation.

20. In step 5, as in step 1c, an exposure assessment via E(N)NDs use should be carried out and levels compared with a HBGV or TTC values. If the intake is lower than the TTC value then the flavouring compound would not be expected to be of health concern. If higher than the TTC value, a risk management decision will need to be taken regarding if it is appropriate for use in E(N)NDs liquids.

References

Elmore, S., Dodson, A. and Mottram, D. (2014) Reactions of Propylene Glycol with the Constituents of Food Flavorings. Proceedings from XIII Weurman Flavour Research Symposium 2014, Flavour science, Pages 577-581.

Erythropel, H.C., Jabba, S.V., DeWinter, T.M.m., Mendizabal, M., Anastas, P.T., Jordt, S.E. and Zimmerman, J.B. (2018) Formation of Flavorant–Propylene Glycol Adducts With Novel Toxicological Properties in Chemically Unstable E-Cigarette Liquids. Nicotine and Tbacco Research, x, 1-11.

Fowles, J. and DiBartolomeis, M. (2017) Toxicological Concerns from Inhaled Food Flavorings Found in Electronic (E-) Cigarette Aerosols. A Report from the Environmental Health Investigations Branch. Exposure Assessment Section, California Department of Public Health.

Zhu, S.-H. and Bonnevie, E. (2014) Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation. Tobacco Control, 23, iii3-iii9.