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TOX/2019/48

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

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 10c: Toxicity assessment of flavourings used in E(N)NDS: Menthol

Background

1. The COT is reviewing the potential human health effects of electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (which, overall, are referred to as E(N)NDS).

2. A number of flavourings are used in E(N)NDS liquids, the toxicity of which has been fully evaluated via the oral route. However, toxicity via inhalation is less widely understood. Two flavourings, vanillin and cinnamaldehyde (TOX/2019/24 and TOX/2019/25) were reviewed at the May 2019 COT meeting. This paper reviews published data on the toxicity via inhalation exposure of menthol, a further flavouring chemical.

Introduction

3. E(N)NDS are battery-powered devices containing a liquid (E(N)NDS liquid or ‘e-liquid’). The E(N)NDS liquid is heated on use to produce an aerosol that is inhaled by the user (‘puffing’, ‘vaping’). E(N)NDS were first introduced commercially in China in 2004 and subsequently in the European Union (EU, 2005) and United States of America (USA, 2007) as nicotine-delivery devices (Bansal and Kim, 2016). The main constituent parts of an E(N)NDS device are a mouthpiece, cartridge (tank) containing E(N)NDS liquid, a heating element/atomizer, a microprocessor, a battery, and sometimes a light-emitting diode (LED) light. Commercially available devices are sometimes categorised as first, second, or third generation. First-generation devices look like conventional cigarettes (CCs) and thus are termed ‘cigalikes’. Initial models comprised three principal parts; a lithium-ion battery, a cartridge and an atomizer. However, more recent models mostly consist of a battery connected to a ‘cartomizer’ (cartridge/atomizer combined), which may be replaceable, but is not refillable. Second-generation E(N)NDS are larger and have less resemblance to tobacco cigarettes. They often resemble pens or laser pointers (hence the name, ‘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

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settings, tank sizes). In addition, highly advanced 'fourth generation' E(N)NDS (innovative regulated mods) are now being described.

4. Constituents that have been identified in E(N)NDS liquids and/or aerosols include PG, VG, water, nicotine, carbonyls, volatile organic compound (VOCs), tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), metals, ethanol, ethylene glycol, di-ethylene glycol, flavouring compounds, flavour enhancers, sweeteners and phenolics.

5. Over 7000 unique flavours of E(N)NDs liquids are reportedly available (Erythropel *et al.*, 2018; Zhu and Bonnevie, 2014), such as green apple, strawberry mint, or caramel cafe.

6. The primary concern about the use of flavouring compounds is that whilst they are approved food flavourings for ingestion in the EU, few have undergone acute or chronic toxicity testing via the inhalation route (Fowles and DiBartolomeis, 2017; Gerloff *et al.*, 2017).

7. Menthol (5-methyl-2-(propan-2-yl)cyclohexan-1-ol) is a popular flavouring agent used in E(N)NDS liquids and cigarette tobacco (DeVito and Krishnan-Sarin, 2018; Leigh *et al.*, 2016). It is a monocyclic terpene alcohol with three asymmetric carbon atoms in the cyclohexane ring, yielding a variety of isomers such as menthol (CAS 89-78-1)[1], L-menthol (CAS 2216-51-5), D-menthol (CAS 15356-70-4) and DL-menthol (CAS 1490-04-6) (figure 1). The L-menthol isomer exhibits the characteristic peppermint odour and flavour and exerts the cooling effects. Other isomers display different taste characteristics and lack the cooling properties. DL-menthol is a synthetic racemic mixture which exhibits approximately half the cooling properties of L-menthol (Heck, 2010). All isomers have been used in E(N)NDS liquids (Bengalli *et al.*, 2017; Leigh *et al.*, 2016; Tierney *et al.*, 2016).

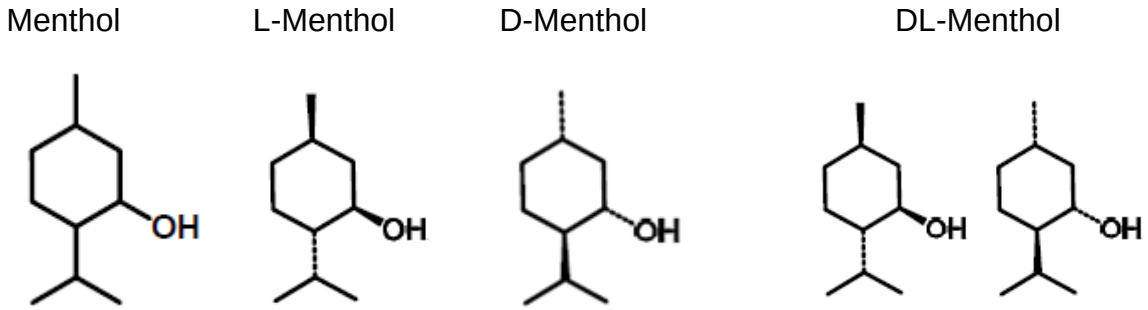

Figure 1 Structure of menthol, L-menthol D-menthol and DL-menthol (SCCS, 2012)

[1] There is some discrepancies regarding the CAS numbers. The CAS numbers in the text are cited in the REACH dossiers and EFSA (2015). However, the SCHEER (2016), SCCS (2012), SCCS (2012) and OECD SIDS (2012) cited menthol (CAS 1490-04-6), DL-menthol (CAS 89-78-1) and L-menthol (CAS 2216-51-5) (OECD SIDS, 2003; SCCS, 2013; SCHEER, 2016). The CAS numbers used in this document are based on those used in the REACH dossiers and the EFSA Opinion.

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8. The following sections summarise data relevant to the inhalation toxicity of the E(N)NDS flavouring compound menthol, including human epidemiological and clinical data and experimental studies in animals.

Search strategies

9. The following search strategies were combined to identify literature relevant to the inhalation toxicity of menthol: 1) Scopus and PubMed databases were searched using combinations of terms as described in Annex A. 2) Reports from authoritative bodies that have reviewed the toxicity and human health effects of exposure to menthol were appraised and relevant literature cited within these reports was identified. 3) Reference lists within the literature citations identified from 1 and 2, above, were inspected for further relevant literature.

Toxicity evaluation

Authoritative reviews

10. Menthol, L-menthol and DL-menthol have been registered under the Registration, Evaluation, Authorisation and restriction of CHemicals (REACH) regulations. They are classified as skin and eye irritants (category 2) (H315: causes skin irritation and H319: causes serious eye irritation). Data were lacking for respiratory sensitisation and effects on or via lactation for menthol and DL-menthol. They are not classified for any other endpoint, including acute inhalation toxicity.

11. D-menthol was listed on the Annex III inventory which was compiled by the European Chemical Agency (ECHA) to identify substances that are likely to meet the criteria of Annex III to the REACH Regulation and thereby be eligible for reduced information requirements to be submitted for registration, rather than a full dataset. D-menthol was considered to meet the ANNEX III criteria due to it being a suspected skin sensitiser (the CAESAR skin sensitisation model in VEGA (Q)SAR platform predicts, with good reliability, that the chemical is a sensitiser), suspected to be toxic for reproduction (the toolbox profiler DART scheme v.1.0 gives an alert for toxicity to reproduction and the CAESAR developmental toxicity model in VEGA (Q)SAR platform predicts that the chemical is a toxicant, with good reliability).

12. Under the Classification, Labelling and Packing (CLP) scheme, industry has notified ECHA that menthol, L-menthol and DL-menthol should be classified according to the following categories: skin and eye irritant (category 2) (H315: causes skin irritation and H319: causes serious eye irritation); Acute Tox. category 4 (H302: harmful if swallowed); and specific target organ toxicity single exposure (STOT SE) category 3 (H335: may cause respiratory irritation). They are not classified for any other endpoint, including acute inhalation toxicity.

13. Menthol is used as a food flavouring and has been designated as GRAS for use in food by the US Flavor and Extract Manufacturers Association (FEMA). FDA has also approved the use of menthol in vapour inhalation products due to its

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antitussive and antipuritic properties. (Heck, 2010; Lee, 2011). Countries that have a regulatory approval framework or have a permitted list of ingredients for use in cigarettes have approved, acknowledged or permitted the use of menthol as a flavouring in cigarettes (Heck, 2010).

14. Several other authoritative bodies have evaluated the toxicity of menthol and isomers via ingestion (EFSA, 2015; FAO/WHO, 1999; FAO/WHO, 2019; SCCS, 2012). However, few assessed the toxicity of menthol and isomers via inhalation.

Acute toxicity

15. An LD50 of 5289 mg/m^3 in male and female rats was reported in the REACH registration dossier for DL-menthol. Five male and female rats per dose were exposed through the nose-only route, to DL-menthol aerosol for four hours (according to OECD 403 – acute inhalation toxicity). Colourless nasal discharge, viscous white content in the nostrils, collapsed lung, bloated stomach and reddened mucosa and red mucous content of the small intestine was reported in treated rats. DL-menthol was used as a read across substance in the registration dossier for menthol and L-menthol, due to a lack of specific acute toxicity data for these (ECHA, 2019a; ECHA, 2019b; ECHA, 2019c).

16. The OECD Screening Information Dataset (SIDS) cited that 'although no experimental data are available for the inhalation route, low systemic toxicity of menthols, based on a LC50 of >2000 mg/kg bw for the oral can also be expected for the inhalation route of exposure' (OECD SIDS, 2003).

Irritation and corrosion

17. Menthol, L-menthol and DL-menthol were tested in four female rabbits, according to OECD 404 – acute dermal irritation/corrosion (Haarmann and Reimer, 1989 cited in OECD SIDS and REACH dossiers). Undiluted and diluted solutions of 50, 25, 5 and 1 % were administered onto shaved skin under semi-occlusive conditions for four hours. Animals were observed for 4.5, 24, 48 and 72 hours, 7 and 14 days. All the undiluted solutions were seen to be irritating to the skin and effects were not reversible within 14 days. Dilution resulted in decreased irritation with no erythema or oedema apparent at 5% menthol and 1% L- and DL-menthol (ECHA, 2019a; ECHA, 2019b; ECHA, 2019c; OECD SIDS, 2003).

18. Menthols also were tested for eye irritation in four female rabbits according to OECD 405 (Haarmann and Reimer, 1989 cited in OECD SIDS and REACH dossiers). L- and DL-menthol, at levels of 28.6 and 64.3 % and menthol[2] at levels between 40 - 100 % were administered to the eye for 24 hours, with further observation for seven days. For all isomers, moderate reactions of cornea (diffuse areas of opacity) and conjunctiva (redness, swelling and discharge) were seen in all rabbits, 1-, 24-, 48- and 72-hours following exposure. No reaction was seen in the

[2] The REACH dossier for menthol cited 40 % solution was used whereas OECD SIDS document cited 71 and 100 % solutions were used.

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iris and all reactions were fully reversible within seven days. The authors noted that the responses seen may not have been substance related, as similar reactions were noted in animals treated with the vehicle only (ECHA, 2019a; ECHA, 2019b; ECHA, 2019c; OECD SIDS, 2003).

19. In a further study in rabbits, undiluted menthol (unknown purity) and 1 and 5 % dilutions caused eye irritation. Overall, irritation was graded 9 out of a maximum of 10. No further details were available (Carpenter and Smyth cited in OECD SIDS, 2003).

20. The potential effect of occupational exposure to menthol vapour during the manufacture of mentholated throat lozenges was evaluated by the National Institute of Occupational Safety and Health (NIOSH). Menthol concentrations in air up to 39.4 mg/m^3 were measured in the production and packaging areas. Inflammation of upper respiratory tissues, rhinitis, lacrimation and ocular redness were reported by employees (no further details available). Pulmonary function testing indicated that within the group of affected individuals, non-smokers and former smokers exhibited significant reductions in forced vital capacity and 1-second forced expiratory volume (FEV1) at the end of a day's workplace exposure, while smokers showed no significant changes in respiratory function in response to menthol vapour. Authors concluded that although there were some limitations to the study such as the study design, it demonstrated that menthol vapour can induce signs of respiratory irritation in some people (NIOSH cited in Heck, 2010). This is similar to a number of other investigations that report exposure to high concentrations of menthol may induce a transient irritation to the skin and mucous membranes (Heck, 2010).

21. Menthol is reported to act on the transient receptor potential (TRP) channels, by being a nonselective agonist of TRP melastatin 8 (TRPM8; also called the cold and menthol receptor), expressed in the primary afferent sensory neurons (Lawrence *et al.*, 2011; Lin *et al.*, 2018; Paschke *et al.*, 2017; Willis *et al.*, 2011). This action is responsible for menthol's cooling, analgesic and counterirritant properties (Journigan et al., 2013 cited in DeVito and Krishnan-Sarin, 2018 and Lin et al., 2018) and affects the sensory impact of nicotine and irritants present in E(N)NDs aerosol. Therefore, the physiological effects of menthol, such as bronchodilation, decreased inhalation rate, antitussive effects, chronic cough or mucus production are predominantly related to the activation of the TRPM8 receptor (Paschke *et al.*, 2017). TRPM8 is also expressed in non-neuronal lung cells including lung epithelial cells, which is the first target in the airways for the direct insult of E(N)NDs aerosol (Lin *et al.*, 2018).

22. A study in mice showed that menthol at a level of 16 ppm in air strongly suppressed respiratory irritation by tobacco smoke as well as other irritants such as acrolein and cyclohexane present in the smoke, whereas a level of 2 ppm showed no effect (Willis *et al.*, 2011). Paschke *et al.* (2017) stated that this was six-fold higher than the estimated minimum menthol concentration required for TRPM8 activation.

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23. Menthol has been shown to increase the sensation of airflow and hinders respiratory activity via DNA damage, masking any reflex actions (coughing). This allows increased lung exposure to cigarette constituents, such as nicotine in e-cigarettes. Consequently, this results in increased lung permeability and absorption of cigarette constituents. As menthol is also known to decrease nicotine metabolism, higher levels of nicotine are therefore maintained in the body (Kaur *et al.*, 2018). Ha *et al*., also concluded that menthol supresses smoke-induced irritation making it easier to inhale smoke and increase the dose of available nicotine (Ha *et al*., 2015 cited in SCHEER, 2016).

24. Aerosolised E(N)NDS-liquids containing different concentrations of nicotine and 0, 0.5 or 3.5 % menthol, or commercial menthol flavours, with and without nicotine were sampled by adult cigarette smokers. At a level of 0.5 % menthol did not affect the perceived irritation/harshness of the E(N)NDS vapour. However, at an inclusion level of 3.5 % menthol, a higher perceived irritation/harshness at low nicotine concentrations but lower irritation/harshness at the higher nicotine concentrations was observed. Authors concluded that 'menthol can reduce perceived airway irritation and harshness produced by inhalation when nicotine concentration is high, and contributes to the sensory impact of E-liquids when nicotine concentration is low' (Rosbrook and Green, 2016).

25. Respiratory sensory irritation is induced by chemical activation of chemosensory receptors in airway-innervating nerves (Erythropel et al., 2018). In a paper addressing the toxicological concerns of food flavourings following inhalation in E(N)NDS aerosols, Fowles and DiBartolomeis (2017) suggested it was necessary to determine the relative irritancy of inhaled flavourings and the potential to cause local irritation to understand their relative toxicity.

26. Respiratory irritants may be ranked according to their RD50, which is the concentration required to reduce the mouse respiratory rate by 50 %. The RD50 has been used to estimate sensory irritancy in animals by a number of authors (Costigan et al., 2014; Erythropel et al., 2018; Kuwabara et al., 2007; Tisserand and Young, 2014).

27. The sensory irritation potential of menthol was assessed in Swiss-Webster mice, exposed to 18-31 ppm (115-198 mg/m^3) menthol for 30 minutes. The RD_{50}, was determined to be 45 ppm (287 mg/m^3). Periocular wetness was observed in several animals 24 h following exposure to concentrations of 22 ppm (140 mg/m^3) and above, and mortalities were recorded among the 20 and 30 ppm (140 and 191 mg/m^3) exposure groups (Burleigh-Flayer, 1988 cited in Heck, 2010).

28. The extent of mucous membrane irritation can be directly related to physico-chemical parameters for chemicals that otherwise have poor toxicological data sets (ECETOC, 2006). For substances from a homologous series, an increased vapour pressure correlated with an increased RD_{50} (Alarie *et al.*, 1995 cited in ECETOC, 2006). A decrease in log octanol-air partition coefficient (K_{ow}) was related to a decrease in RD_{50}, thereby both could be used as a predictor of the severity of

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sensory irritation (ECETOC, 2006). The ECETOC Task Force derived a relationship to predict the RD_{50} from the air-water partition coefficient (K_{aw}) and the K_{ow} using the equation below.

$$\text{Log RD50} = b_0 + b_1 \times \log K_{ow} + b_2 \times \log K_{aw}$$

Where:

b0=6.346; b1=-0.8333; b2=0.7139

29. Using the equation above, the calculated RD_{50} for menthol, L-menthol and DL-menthol would be 17, 27 and 8 ppm, respectively.

Sensitisation

30. A number of sensitisation studies have been reported with isomers of menthol. A Buehler test was carried out in guinea pigs with L-menthol, according to OECD 406. Twenty female guinea pigs were administered 0.5 mg of 25 % solution of L-menthol under occlusive conditions during the induction and challenge phase of the test. No positive reactions were reported (Haarmann and Reimer, 1991 cited in ECHA, 2019b and OECD SIDS 2003).

31. A local lymph node assay (LLNA) was carried out in mice according to OECD 429. Four male mice per dose were administered 25 µl of 1, 10 or 30 % L-menthol and did not exhibit any sensitising effects (Haarmann and Reimer, 1991 cited in ECHA, 2019b and OECD SIDS 2003).

32. In humans, a maximisation test with 8 % DL-menthol in petrolatum was carried out in 25 volunteers. No positive reactions were reported (no further details available) (Kligman, 1975 cited in Opdyke, 1976 and OECD SIDS, 2003).

33. OECD SIDS (2003) cited that 'The presence of menthol and menthol-containing flavour and fragrance oils in consumer products such as cigarettes, toothpaste, and topical medications can lead to sensitivity reactions in the oral and nasal cavity of susceptible persons. However, based on the wide exposure of consumers to these substances and also on the results from clinical studies, which investigated a high number of subjects, the overall sensitizing potential of the menthol isomers is considered to be low'.

34. A number of patch-test studies have also been reported in human patients. Allergic contact hypersensitivity was investigated in a group of 228 dermatology patients, by patch testing with 1 % menthol. Sensitisation was observed in 1.3 % of patients. In a group of 330 patients with eczemous lesions tested with 1 % menthol 6.1 % showed positive patch tests and in a study of 1385 eczema patients tested with 1 % menthol, 6 patients (0.4 %) showed allergic reactions including dermatitis, eczematous lesions and dermatoses (Baer et al., 1955; Bloneel et al., 1978; Jarisch and Sandor, 1978 cited in OECD SIDS, 2003 and ECB, 2000). In a longer-term study, 5 % menthol and peppermint oil was administered to 512 dental patients with interoral symptoms over a four year period to assess the potential to stimulate

contact sensitivity. Ten patients demonstrated contact sensitivity to menthol on day 4 and their symptoms resolved when menthol exposure was avoided (Morton et al., 1995 cited in ECB, 2000 and SCCS, 2012).

Repeat dose toxicity

35. Mice (strain, sex and number not reported) were administered 0.05 or 0.1 mg/L menthol via inhalation (type of inhalation unknown) for three months (no further details available). The authors of the REACH registration dossier noted 'regressive changes' in the liver and kidney, representing symptoms of the chronic intoxication. A no observed adverse effect concentration (NOAEC) of 10 mg/L was reported by the authors, however it is unclear how this was determined as the maximum concentration administered was 0.1 mg/L (Kowalski et al., 1962 cited in ECHA, 2019a).

36. Six male and female Sherman rats per group were exposed to L-menthol by whole body vapour inhalation for 71 to 79 days (no further details available) (Rakieten et al., 1954 cited in OECD SIDS, 2003 and Belsito et al., 2008). At the time the study was carried out, no analytical methods were available to measure the exposure concentrations of menthol hence these were determined by dividing the weight of menthol vaporised by the circulating air volume. This gave estimated concentrations of 0.087, 0.148 and 0.259 ppm (0.57, 0.96 and 1.368 mg/m^3). No gross toxic effects were found. Histopathological organ examinations showed evidence of lung toxicity, ranging from tracheitis to severe congestion of the lungs only at the highest dose, indicative of irritation. OECD SIDS (2003) concluded that this identifies the respiratory system as a possible target organ after exposure by inhalation.

37. Vanscheeuwijk *et al*. (2002) carried out a 90-day inhalation study in rats according to OECD 413 (Subchronic inhalation toxicity: 90 day study) using an American-style non-menthol reference cigarette and a similarly blended test cigarette containing 5000 ppm L-menthol. Male and female Sprague-Dawley rats (10/sex/dose) were nose only exposed to mainstream smoke particulate concentrations of 200, 600 or 1200 mg/m^3 for one hour per day, five days per week for 90 days. There were no differences in the pathology of the respiratory tract in the different dose groups. Authors concluded that the addition of 5000 ppm menthol to tobacco had no substantial effect on the character or extent of the biological responses normally associated with the inhalation of mainstream cigarette smoke in rats (Gaworski *et al.*, 1997).

38. Another 90-day inhalation study was carried out by Vanscheeuwijck *et al.* (2002). Ingredients commonly used in the manufacturing of cigarettes were added to cigarettes at a 'low and high level'. The low level reflected levels in modern cigarettes and the high level was 1.5 or 3 times higher than the low level. However, for menthol, the same concentration was used in both cigarettes as it was not possible to add more menthol into the cigarettes. Groups of ten male and female Sprague-Dawley rats were nose only exposed to 150 µg total smoke particulate

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matter/litre air for six hours per day seven days per week for 90 days. Authors concluded that toxicity of the smoke of the menthol-containing test cigarette did not appear to differ in any substantive way from that of the non-menthol reference cigarette.

39. Baker et al., (2004) also carried out 90-day studies using three series of test cigarettes. Twenty-two groups of animals were exposed to smoke from various test and control cigarettes as well as air control groups. The maximum concentration of menthol tested was 23,400 ppm (no further information regarding the concentrations was provided). Groups of ten male and female Sprague-Dawley rats were nose-only exposed to target levels of 1 mg total particulate matter/litre air, for one hour per day (smoke 30 min, air 15 min, smoke 30 min, for 5 days per week) for 90 days. The target levels were selected according to the potential human exposure, existing toxicity data and any limitations imposed by the exposure apparatus and procedure as well as the stability of the experimental atmosphere. There were no differences in toxicity of menthol cigarettes compared to non-menthol cigarettes.

Mutagenicity/genotoxicity

40. L-Menthol was not mutagenic in Ames tests using S. typhimurium TA97a, TA98, TA100, TA102, TA1535, TA1537, and TA2637 with and without metabolic activation (Nohmi *et al*.,1985, Andersen and Jensen, 1984, Gomes-Cameiro, et al., 1998 cited in OECD SIDS, 2003 and Belsito et al., 2008). Some tests were carried out at cytotoxic concentrations (800 µg/plate; Gomes-Carneiro, et al., 1998). A reverse mutation assay with E. coli WP2 uvrA (trp-) was also negative at concentrations up to 800 µg/plate (Yoo et al., cited in OECD SIDS, 2003).

41. Peripheral blood lymphocytes from 24 human donors were treated with 0.1-10 mM L-menthol with and without S9 mix. No chromosomal aberrations were noted. Similarly, no chromosomal aberrations were seen in human fibroblasts treated with 0.1 to 10 µg/ml L-menthol. Chromosomal aberration tests were also carried out with Chinese hamster lung cells (CHL), treated with 0.1 to 0.3 mg/ml L-menthol. Tests were negative with and without metabolic activation (Murthy et al., cited in FDA, 1975, Belsito et al., 2008 and OECD SIDS, 2003).

42. DL-Menthol was not mutagenic in Ames tests using S. typhimurium TA97a, TA98, TA100, TA102, TA1535, TA1537, and TA2637 with and without metabolic activation and when treated with concentrations that caused cytotoxicity concentrations (Nohmi et al., 1985; Ishidate et al., 1984; Zeiger et al., 1988 cited in Belsito et al., 2008 and OECD SIDS, 2003).

43. A mouse lymphoma assay, carried out with L5178Y cells treated with 12.5 to 200 µg/ml DL-menthol, with and without metabolic activation, was negative (OECD SIDS, 2003). In addition, an alkaline elution assay to detect DNA damage in primary rat hepatocytes was also negative. Concentrations of 0.1, 0.3, 0.7, 1 and 1.3 mM up to cytotoxic concentrations were tested (Myhr and Caspary, 1991 cited in Belsito et al., 2008 and OECD SIDS, 2003).

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44. A number of chromosomal aberration assays were carried out. Tests with Chinese hamster ovary (CHO) cells, treated with concentrations up to 200 µg/ml with and without metabolic were negative. CHL cells tested with concentrations up to 200 µg/ml were also negative without metabolic activation. In contrast, weak but statistically significant positive results were reported in CHO cells and TK6 human lymphocytes treated with 250 or 281 µg/ml DL-menthol, without metabolic activation. However, this result could only be reproduced for the highest scorable concentration of CHO cells when the test was repeated (Ivett et al., 1989, Sofuni et al.,1985 and Ishidate et al.,1984 and Hilliard et al., 1998 cited in OECD SIDS, 2003).

45. *In vivo*, chromosomal aberrations were not increased in the bone marrow of rats exposed to L-menthol via oral exposure at a single dose of 3000 mg/kg bw or 5 doses of 1150 mg/kg bw/day. This assay was carried out in accordance with current standards (OECD SIDS, 2003). L-menthol was also not mutagenic in a dominant lethal test in rats treated with a single dose of 1.45, 14.5, 145, 500 or 3000 mg/kg bw (14 to 20 pregnant females per mating group) or 5 doses of 1.45, 14.5, 145 or 1150 mg/kg bw/day (13 to 19 pregnant females per mating group) (FDA, 1975 cited in OECD SIDS, 2003).

46. DL-menthol was administered to B6C3F1 mice daily via intraperitoneal injection of 250, 500 or 1000 mg/kg bw/day D/L-menthol for 3 days in a micronucleus assay. No increase in micronuclei was observed in bone marrow cells. However, the negative result was considered to be of limited relevance as no toxicity to the bone marrow was observed. Testing at higher doses was not possible due to 50 % mortality occurring at the highest dose tested (Shelby et al., cited OECD SIDS, 2003).

47. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that there are no structural alerts for genotoxicity for menthol and DL-menthol hence they are unlikely to be genotoxic based on the weight of evidence (JECFA, 2019).

Carcinogenicity

48. Lee (2011) carried out a systematic review of epidemiology studies and identified eight studies that reported relative risk of lung cancer associated with the use of mentholated cigarettes. The studies included one case-control study from Germany, five case-control studies from the USA and two prospective cohort studies from the USA. Authors stated that the eight studies had valid cases and controls, and appropriate adjustment for age, gender, race and smoking. However, only one study presented data by histological type, none adjusted for occupation or diet and some did not provide information on length of mentholated cigarette use. They concluded that although some weaknesses exist, the epidemiological evidence is consistent with previous data that show that mentholation has no effect on the lung carcinogenicity of cigarettes.

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49. No experimental carcinogenicity studies were found relating to the inhalation of menthol or its isomers in animals.

50. DL-menthol was not carcinogenic in Fischer 344 rats or B6C3F1 mice (50 animals per sex and dose) treated for 103 weeks with 3750 or 7500 ppm (188 and 375 mg/kg bw/day for rats and 334 and 667 mg / kg bw/day for mice) in feed. The study was carried out according to OECD 453 (NCI, 1979). Since DL-menthol contains D- and L- isomers in a 50:50 ratio it can be assumed that D- and L-menthol also are not carcinogenic (OECD SIDS, 2003),

51. A SENCAR mouse skin painting assay using smoke condensate from menthol and non-menthol cigarettes did not show any significant adverse effect of menthol (Baker et al., 2004 in FDA, date unknown).

Reproductive and developmental toxicity

52. No inhalation route specific reproductive or developmental studies were found for menthol or its isomers in animals or humans. An extended one-generation reproductive dietary study in rats (cohorts 1A and 1B without extension) has been proposed by the registrant (ECHA, 2019a).

53. Oral repeat dose and carcinogenicity studies, carried out according to OECD 453, showed that DL-menthol did not affect the reproductive organs of rats and mice (OECD SIDS, 2003).

54. Various developmental studies have been carried out with L-menthol in rats, hamsters, mice and rabbits. No treatment related effects were reported following administration via oral gavage on gestation day 6 to 10, 15 or 18 (ECHA, 2019a; ECHA, 2019b; ECHA, 2019c).

Other

55. A number of papers that indicate an effect of menthol on nicotine dependence have been identified.

56. Menthol has been demonstrated to facilitate dependence or worsen cessation outcomes in some groups of smokers. Five out of ten studies showed a significantly worse cessation outcome in menthol vs non-menthol smokers. The remaining five studies did not show a significant difference between the groups (Foulds et al., 2010 cited in DeVito and Krishnan-Sarin, 2018).

57. Following a literature review of studies between 2002 and 2010, authors reported menthol was associated with increased nicotine dependence in cigarette smokers, measured by shorter time to first cigarette following waking, lower quit rates and higher relapse rates, compared to non-menthol smokers (Ahijevych et al., 2004 cited in DeVito and Krishnan-Sarin, 2018).

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Thermal decomposition of menthol

58. During E(N)NDS use, the vaporisation temperature has been estimated to be above 40 °C. The heating period introduces the potential for pyrolysis of compounds. Therefore, thermal degradation and reaction products of flavourings should also be considered in the assessment of risk (Costigan and Meredith, 2015).

59. In their Final Opinion on Additives Used in Tobacco Products, SCENIHR (2016) noted that 'thermal decomposition or burning may release carbon monoxide or other hazardous gases, acrid smoke and irritating fumes' and concluded that 'data on pyrolysis of most of the individual additives are scant' and called for more pyrolysis studies on individual and complex flavour additives to be carried out. It should be noted that CCs generally reach higher temperatures compared to E(N)NDS, hence the pyrolysis profile may be different. The FDA Centre for Tobacco products (CTP) also considered extensive pyrolysis, smoke chemistry and biological evidence in its evaluation of menthol in cigarettes (Czégény *et al.*, 2016; FDA).

60. Menthol is reported to be converted to menthone, mentene and menthane on heating (SCHEER, 2016a). 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.

Summary

61. There are many different varieties of E(N)NDs liquids on the market made up of a number of flavouring chemicals, as well as PG, VG, nicotine and water. Few of these flavourings have undergone acute or chronic toxicity testing via the inhalation route. Therefore, the potential toxicity via E(N)NDs use cannot currently be ascertained.

62. 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.

63. The respiratory sensory irritation potential of menthol has been investigated using a number of approaches. The RD_{50} was measured in mice and also calculated based on physico-chemical parameters (see paragraph 27). Menthol is known to activate TRPM8 receptors which is responsible for its cooling, analgesic and counterirritant properties; this may also affect the sensory impact of nicotine and irritants in E(N)NDS aerosol.

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64. 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 animals that assessed either exposure to menthol or to cigarette smoke containing menthol. No adverse effects were observed that could be attributed to menthol.

65. No reproductive or developmental studies could be identified that addressed with the impact of menthol via the inhalation route on these endpoints.

Questions for the Committee

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

i. Are there any data gaps with respect to the risk assessment for menthol or other particular aspects of this paper which should be captured in the COT statement on E(N)NDS?

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

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Abbreviations/Glossary

CC	Conventional Cigarettes
CHO	Chinese Hamster Ovary
CHL	Chinese Hamster Lung
CLP	Classification, Labelling and Packing
CTP	Centre for Tobacco products
E(N)NDS	Electronic Nicotine and Non-Nicotine Delivery Systems
ENDS	Electronic Nicotine Delivery Systems
ENNDS	Electronic Non-Nicotine Delivery Systems
EU	European Union
FDA	US Food and Drug Administration
FEMA	Flavour Extracts Manufacturers Association
FEV1	1-second forced expiratory volume
GRAS	Generally Regarded As Safe
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K_{aw}	Air-Water Partition Coefficient
K_{ow}	Octanol-Air Partition Coefficient
LC_{50}	The concentration that is lethal to 50 % of a test population
LD_{50}	The dose that is lethal to 50 % of a test population
LED	Light-Emitting Diode
LLNA	Local Lymph Node Assay
NIOSH	National Institute of Occupational Safety and Health
NOAEC	No observed adverse effect concentration
PAH	Polycyclic Aromatic Hydrocarbon
PG	Propylene Glycol
RD_{50}	The concentration required to reduce the mouse respiratory rate by 50%
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
SIDS	Screening Information Dataset
STOT SE	Specific target organ toxicity single exposure
TRP	Transient receptor potential
TRPM8	Transient receptor potential melastatin 8
TSNA	Tobacco-Specific Nitrosamine
VG	Vegetable glycerol
VOC	Volatile Organic Compound
WHO	World Health Organization

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

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 10c Toxicity assessment of flavourings used in E(N)NDS: Menthol

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

Relevant literature was obtained from reviews published by authoritative bodies, as described in paragraph 4 of the main report. In addition, searches for further literature relating to toxicity of E(N)NDS aerosol were identified as described below. The following three sets of literature searches were performed by NCET at WRc/IEH-C under contract to PHE on xxx in Scopus and PubMed, with no limit of publication date.

Search 1: toxicity

Scopus

((CASREGNUMBER ("1490-04-6" OR "89-78-1") OR CHEMNAME (menthol) OR TITLE-ABS-KEY (menthol))) AND ((TITLE-ABS-KEY (*toxic* OR acute OR irritation OR sensitization OR "repeat dose" OR carcin* OR mutagen*) AND TITLE-ABS-KEY (inhal*))) AND (LIMIT-TO (LANGUAGE , "English")) AND (EXCLUDE (LANGUAGE , "German")):68

PubMed

((("1490-04-6" OR “89-78-1”[EC/RN Number]) OR menthol[Title/Abstract])) AND (((*toxic* [Title/Abstract] OR acute [Title/Abstract] OR irritation [Title/Abstract] OR sensitization [Title/Abstract] OR "repeat dose" [Title/Abstract] OR carcin* [Title/Abstract] OR mutagen*[Title/Abstract])) AND inhal*[Title/Abstract]) AND english[Language]: 31

Search 2: thermal degradation

Scopus

((CASREGNUMBER ("1490-04-6" OR “89-78-1”) OR CHEMNAME (menthol) OR TITLE-ABS-KEY (menthol))) AND (TITLE-ABS-KEY ("thermal decomposition" OR "thermal breakdown" OR "thermal degradation" OR thermolysis)): 14

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PubMed

((("1490-04-6" OR “89-78-1”[EC/RN Number]) OR (menthol[Title/Abstract]))) AND (("thermal decomposition" [Title/Abstract] OR "thermal breakdown" [Title/Abstract] OR "thermal degradation" [Title/Abstract] OR thermolysis[Title/Abstract])): 0

For completeness, the reference lists of selected papers were examined for further relevant publications, and additional *ad hoc* searches were carried out as considered appropriate.