TOX/2019/25

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 10b: Toxicity assessment of flavourings used in E(N)NDS: Cinnamaldehyde

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, may also be referred to as E(N)NDS). A summary of publications describing the chemical constituents of E(N)NDS liquids and aerosols (excluding metals and flavourings) (TOX/2018/16) was presented at the COT meeting in March 2018. A review of published data on the toxicity of the major constituents of E(N)NDS liquids such as propylene glycol (PG) and glycerol (VG) (TOX/2018/19) was presented at the COT meeting in May 2018, and a paper reviewing published data on the toxicity of E(N)NDS aerosols (TOX/2018/24) and nicotine (TOX/2018/25) were presented at the July 2018 COT meeting.

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. This paper reviews published data on the toxicity of one such flavouring chemical, cinnamaldehyde, via inhalation exposure.

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

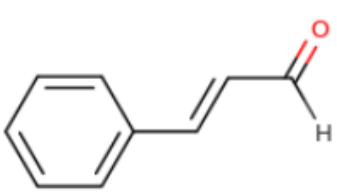
cigarettes. They often resemble pens or laser pointers (hence the name, 'vape pens'). They have a high-capacity rechargeable lithium-ion battery and a refillable atomizer (sometimes referred to as a 'clearomizer'). Third-generation models ('advanced personal vapers', 'mods') are also refillable, have very-high-capacity lithium-ion batteries and are highly customisable (different coil options, power 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 compounds (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. E(N)NDS liquids are comprised of flavouring chemicals, such as vanillin or cinnamaldehyde, with PG, VG, nicotine and water, hence flavouring compounds are one of the five most commonly listed ingredients in E(N)NDS liquids. The primary concern about the use of flavouring compounds is that whilst they are approved food flavourings for ingestion and have been deemed 'generally regarded as safe (GRAS)' by the US Food and Drug Administration (FDA) or World Health Organization (WHO), few have undergone acute or chronic toxicity testing via the inhalation route (Fowles and DiBartolomeis, 2017).

6. Cinnamaldehyde is a popular flavouring agent used in E(N)NDS liquids (Clapp et al., 2019; Erythropel et al., 2018). Cinnamaldehyde (cinnamal/3-phenyl-2-propenal, CAS 104-55-2) occurs naturally in the bark of cinnamon trees. The configuration of the double bond in cinnamaldehyde has yet to be specified, however, it is anticipated to contain more than 97 % trans-cinnamaldehyde (CAS 14371-10-9) (EFSA, 2009a). The structure of cinnamaldehyde is given in figure 1.

Cinnamaldehyde
CAS 104-55-2



Trans-cinnamaldehyde
CAS 14371-10-9

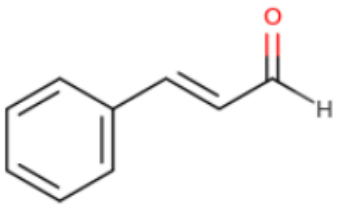


Figure 1. Structure of cinnamaldehyde

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

Search strategies

8. The following search strategies were combined to identify literature relevant to the inhalation toxicity of cinnamaldehyde: 1. Scopus and PubMed databases were searched using combinations of terms as described in Annex A. Reports from authoritative bodies that have reviewed the toxicity and human health effects of exposure to vanillin 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

9. Cinnamaldehyde has been registered under the Registration, Evaluation, Authorisation and restriction of CHemicals (REACH) regulations. It is classified as a skin irritant (category 2), eye irritant (category 2) and a skin sensitiser (category 1) and has the hazard statements H315: causes skin irritation, H319: causes serious eye irritation and H317: may cause an allergic reaction. It was not classified for any other endpoint, including acute inhalation toxicity. The acute inhalation classification was based on a quantitative structural analysis relationship (QSAR) prediction using the Organisation for Economic Co-operation and Development (OECD) toolbox v2.3 (see acute toxicity section for details).

10. Several authoritative bodies have evaluated the toxicity of cinnamaldehyde via ingestion, including Scientific Committee on Consumer Safety (SCCS, 2012), Scientific Committee On Cosmetic Products And Non-Food Products Intended For Consumers (SCCNFP, 1999), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1995), European Food Safety Authority (EFSA, 2009a; EFSA, 2009b), National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2016), New Zealand Environmental Protection Agency (NZ EPA, Date unknown), Danish Environmental Protection Agency (Danish EPA, 2016), United States Environment Protection Agency (US EPA, 2008), Research Institute for Fragrance Materials (RIFM)(Bickers et al., 2005)) and WHO (JECFA, 1980; WHO, 2001). However, none assessed the toxicity of cinnamaldehyde via inhalation.

Acute toxicity

11. No experimental LC_{50} data were found for cinnamaldehyde.

12. In the REACH dossier, an LC_{50} of 68.88 mg/l was predicted using the OECD QSAR toolbox. This was calculated for male and female Wistar rats, exposed to cinnamaldehyde vapour for 4 hours via inhalation. The prediction was based on a dataset comprised from the following descriptors: LC_{50}, estimation method taking

average value from the nearest six neighbours and the prediction was in domain. Authors concluded that, based on the prediction, cinnamaldehyde was not toxic through the inhalation route (ECHA, 2019).

Irritation and corrosion

13. Limited animal data are available on respiratory irritation. In humans, inhalation of nebulised cinnamaldehyde (125-800 mM) resulted in irritation of the upper airways (coughing) in all ten subjects exposed in a single study. Authors noted a dose-response relationship, the response being the number of coughs following exposure. Cinnamaldehyde was identified as a specific agonist of the TRPA1 (transient receptor potential A1) receptor and induced coughing due to chemaesthesis of the airways (NICNAS, 2016). Mucous membranes may also become irritated following exposure to high concentrations of inhaled cinnamaldehyde which may lead to coughing. No further information is available (Garcia and Harbison, 2015).

14. Respiratory sensory irritation is the first sign of potential toxicity of an inhaled chemical, which 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 the relative toxicity.

15. Respiratory irritants may be ranked according to their RD_{50}, which is the concentration required to reduce the mouse respiratory rate by 50 %. The RD_{50} 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). Tisserand and Young (2014) reported that RD_{50} values correlate well with log lowest observed adverse effect levels (LOAELs) in humans, suggesting that RD_{50} is a useful predictor of safe public exposure levels. The RD_{50} has been successfully correlated with irritant thresholds in occupational and general population settings (Alarie 1986; Alarie et al., 1995 and Kuwabara et al., 2007 cited in ECETOC, 2006) and is accepted as a standard measure of sensory irritation. .

16. The irritant properties of inhaled food additive aldehydes in mice, using the RD_{50} as a ranking metric has been reported (Steinhagen and Barrow, 1984 cited in Fowles and DiBartolomeis (2017)). In vitro tests quantifying the capability of a chemical to activate TRP irritant receptors are currently being considered as replacements for animal studies to determine the RD_{50}. Recent studies identified TRP ion channels TRPA1 and TRPV1 to be the receptors for irritant aldehydes in airway-innervating nerves. They are activated by flavour aldehydes such as cinnamaldehyde, eliciting irritation responses, pain, and cardiovascular reflexes increasing stress and inflammation (Bautista et al., 2006; Richards et al., 2010; Achanta et al., 2017 and Pozsgai et al., 2010 cited in Erythropel et al. (2018)).

Cinnamaldehyde activated TRP irritant receptors in HEK-293T cells suggesting that it may act as an airway irritant in e-cigarette users (Erythropel et al., 2018).

17. As well as determining the RD_{50} from animal data or in vitro data, 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 the 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.

$$\text{Log } RD_{50} = b0 + b1 \times \log K_{ow} + b2 \times \log K_{aw}$$

Where: b0=6.346; b1=-0.8333; b2=0.7139. b0, b1 and b2 were estimated by multiple regression.

18. Using the equation above, the calculated RD_{50} for cinnamaldehyde would be 68.25 ppm.

19. 0.03 x RD_{50} may be considered to be the threshold for irritation in humans (Fowles and DiBartolomeis, 2017; Kuwabara et al., 2007; Tisserand and Young, 2014). Fowles and DiBartolomeis (2017) suggested that flavourings, many of which are found in E(N)NDS liquids, would qualify as “moderate” irritants if the RD_{50} was <1000 ppm and concluded that ‘it may be useful to establish a mechanism to classify and categorise the flavouring chemicals for their potential respiratory irritancy whether or not specific respiratory irritation data exist for each individual chemical. The use of the calculated RD_{50} based on physical properties of the individual chemical [as presented in this paper], may be one way to accomplish this’.

20. Many studies have been carried out to assess the skin irritation potential of cinnamaldehyde, both in animals and humans. In most cases it is unknown whether they were carried out according the OECD test guidelines or under GLP.

21. The RIFM expert panel evaluated a number of in vivo studies. Cinnamaldehyde caused mild skin irritation in mice and guinea pigs at concentrations of 3-5 % and was non-irritating to rabbits at 1 %. Severe erythema, eschar and light to moderate edema was seen in rabbits (10, 2-4 and 10 rabbits) in LD_{50} studies with 100 % (undiluted) cinnamaldehyde (Bickers et al., 2005).

22. Cinnamaldehyde was tested on four rabbits (100 % cinnamaldehyde, 0.5 ml) for four hours. Yellow staining of fur was observed in all animals tested. ‘Well-defined’ erythema was observed after 1 hour in all animals. Irritation remained throughout the seven day observation period in 2 out of 4 animals but was reversible in 1 animal. The fourth animal died. No information was given regarding the cause of death. Similarly, severe oedema was seen in all animals after 1 hour, which in

general, was reversible during the observation period. Based on the data the primary irritation index (PII) was 3.71 (ECETOC, 1995).

23. NZ EPA cited a guinea pig study in which animals showed irritation following exposure to 0.5 % cinnamaldehyde in vaseline and 1 % in acetone. No further information is available (NZ EPA, Date unknown). US EPA also considered cinnamaldehyde to be a strong skin irritant to guinea pig skin (no further details available) (US EPA, 2008).

24. In humans, cinnamaldehyde did not produce irritation in 171 volunteers exposed to 0.125–1.25 %, but was classed as irritating in 10/63 volunteers at 3 % and severely irritating in 5/5 volunteers at 8 % (Bickers et al., 2005).

25. NICNAS (2016) also reported a number of human studies, including those cited by Bickers et al. (2005). In another study, doses of 0.02, 0.1 and 0.8 % cinnamaldehyde in ethanol were applied to human skin for six weeks. The chemical was concluded to be severely irritating to human skin. This study was used as the key study in the REACH dossier.

26. The RIFM expert panel reported eye irritation in rabbits following exposure to 0.125, 1 and 1.25 % cinnamaldehyde, which caused irritation, mild irritation and intense irritation, respectively (Bickers et al., 2005). Cinnamaldehyde was also moderately irritating in rabbit eyes, which was reduced by washing the eyes. No further details are available (US EPA, 2008).

Sensitisation

27. A number of sensitisation studies have been reported with cinnamaldehyde, both in humans and animals.

28. Cinnamaldehyde is a well-recognised and frequently reported contact allergen (IFRA, 2013; SCCNFP, 1999; SCCS, 2012). It comprises one of the eight components of the fragrance mix used in diagnostic tests to determine allergenicity to common chemicals used in fragrances.

29. A number of human repeat insult patch tests (HRIPTs) have been carried out in humans to determine the sensitisation potential both in healthy volunteers as well as those with suspected allergies.

30. The SCCNFP reported that cinnamaldehyde accounts for 5–36 % of the reactions to the fragrance mix used in diagnostic tests (SCCNFP, 1999). Moreover, 0.5 % cinnamaldehyde induced reactions in 2-3.7 % of consecutively patch tested patients and a level of 1 % has been shown to cause allergic reactions in patch tests in 1-30 % of patients with eczema following exposure to cosmetic products. Skin irritation effects were generally predominant at concentrations above 3 % cinnamaldehyde, and often impeded the interpretation of results from the patch testing (SCCNFP, 1999). Although fewer cases of sensitisation in humans were

reported at <1 % cinnamaldehyde, some positive allergic responses were observed with 0.2 % cinnamaldehyde (Cocchiara et al., 2005 cited in NICNAS (2016)).

31. The RIFM expert panel evaluated 42 sensitisation tests carried out in guinea pigs or mice, including Magnusson–Kligman maximization tests, Modified Draize tests, Buehler Delayed Hypersensitivity tests, Maguire tests, Freunds Complete Adjuvant Test, Closed Epicutaneous Test, Open Epicutaneous Test, Cumulative Contact Enhancement Test and the Local Lymph Node Proliferation Assay, at concentrations from 0.1 to 40 % in various vehicles. In general, sensitisation was observed at all dose levels tested and in almost every study (Bickers et al., 2005).

32. The SCCS cited a number of local lymph node assays (LLNA). The lowest EC3 value (concentration required to provoke a 3-fold increase in lymph node cell proliferative activity compared with controls) from all studies was 0.2 %. This was calculated from an in vivo mouse LLNA study equivalent to OECD TG 429. Cinnamaldehyde was administered to mice (4/group) at concentrations of 0, 0.1, 0.3, 1, 3 or 10 % (w/v) in ethanol/diethyl phthalate (ratio of 3:1) causing positive reactions. No further information is available (SCCS, 2012). A similar study using 0, 0.5, 1, 2.5, 5 or 10 % (w/v) in acetone/olive oil (ratio 4:1) reported skin sensitisation reactions. The EC3 was 3.2 % (Basketter et al., 2001 cited in NICNAS (2016)).

33. A recent review of cinnamaldehyde by the Danish EPA reported data for LLNA and guinea pig maximisation tests (GPMTs). Nineteen LLNA studies reported EC3 values of ≤2 % and 1 study > 2 %. Four GPMTs were cited, all of which reported that cinnamaldehyde was a sensitiser (Danish EPA, 2016). US EPA also considered cinnamaldehyde to be a moderate skin sensitiser in guinea pigs (no further details available) (US EPA, 2008).

34. ECETOC also cited that positive responses were recorded in GPMTs, and in the Buehler test as well as the LLNA in mice and the mouse ear swelling test. They concluded that cinnamaldehyde is an animal skin sensitiser and would be expected to elicit clear positive reactions in a predicted test for skin sensitisation (ECETOC, 1999).

35. Sensitisation following cinnamaldehyde exposure was also reported in a non-invasive mouse ear swelling assay that was designed for evaluating fragrances and mixtures. No further information available (Garcia and Harbison, 2015).

36. An early study reported that formation of a cinnamaldehyde-protein conjugate in the skin due to cinnamaldehyde reacting with nucleophilic groups in proteins is considered to be responsible for immunogenicity observed. The binding sites on the protein appear to be mostly thiol groups of cysteine residues (Weibel and Hansen, 1989).

37. The allergic reactions to cinnamaldehyde have been related to its Michael reactivity and its ability to form stable adducts with proteins. This is similar to the mechanism by which cinnamaldehyde activates TRPA1, by alkylation of the thiol-rich

ankyrin moiety. Although it is widely accepted that cinnamaldehyde reacts with thiol groups, the exact mechanism how it traps thiols is unclear (Auteliano et al., 2017).

Repeat dose toxicity

38. No repeat dose inhalation toxicity tests on cinnamaldehyde were found.

39. Coggins et al. (2011) evaluated a number of aromatic carbonyl compounds, using mainstream smoke from four experimental cigarettes, containing differing concentrations of cinnamaldehyde. The target inclusion levels for controls, low, and high levels were 0, 100, 1000 and 10,000 ppm, respectively. No method was available to quantify the levels in tobacco hence levels in tobacco before cigarette production, in cigarettes after production and post-analysis could not be measured, which was noted by the authors as a limitation of the study. Cinnamaldehyde did not adversely affect cytotoxicity (expressed as $1/EC_{50}$, where an EC_{50} is the concentration required to produce an effect in 50 % of a test population) at any concentration. Authors stated that compounds with inclusion levels <100 ppm were not tested using the inhalation study, hence cinnamaldehyde was not tested in the inhalation test in rats.

Mutagenicity/genotoxicity

40. Cinnamaldehyde contains an α,β-unsaturated aldehyde group which is a common structural alert for genotoxicity due to the ability to form DNA adducts (NICNAS, 2016). A number of assays have been carried out including a chromosomal aberrations test, sister chromatid exchange test, in vivo micronucleus assay, Ames test and unscheduled DNA synthesis assay.

41. Cinnamaldehyde (100 µl/plate) was negative in the Ames test in S. typhimurium strains TA97, TA98, TA1335 and TA1535, with and without metabolic activation and was positive in TA100 in 2 out of 11 tests. It was also negative in other mutation assays in E. coli strain WP2 uvra (600 and 800 µl/plate) and in V79 cells (0.05 to 5000 µl/plate) both with and without metabolic activation. Sister chromatid exchange tests in Chinese ovary cells were negative at low concentrations but weakly positive at higher concentrations although cytotoxicity was also reported. Positive results for chromosomal aberrations were also reported in Chinese hamster lung fibroblasts and B241 cells at low concentrations (<15 µg/ml), with and without metabolic activation but not in Chinese ovary cells or human HAIN-55 fibroblast cells treated with higher concentrations (up to 100 µg/ml).

42. NICNAS also evaluated various in vivo studies which have been carried out including a bone marrow micronucleus assay in mice following i.p. administration; micronucleus assay in rats and mice following oral gavage; and unscheduled DNA synthesis or S-phase synthesis in mice and rats following oral administrations. All were negative with the exception of micronuclei in hepatocytes and forestomach mucosal cells, although such results were attributed to the bolus doses resulting in high exposure of the liver and stomach. Overall, based on evaluation of the in vitro

and in vivo experimental data, NICNAS considered cinnamaldehyde not to be genotoxic (NICNAS, 2016).

43. EFSA cited text from JECFA (WHO, 2001) that based on various studies, it was concluded that 'cinnamyl alcohol and related compounds lack direct mutagenic or genotoxic activity, as indicated by the negative results obtained in bacterial test systems. The mixed results in the assay for DNA repair and in various studies of antimutagenicity were associated with cytotoxicity. Evidence of genotoxic activity was found in isolated mammalian cells, the cinnamyl compounds inducing chromosomal aberrations and/or mutations in the presence or absence of metabolic activation; however, the reported activity in vitro was not seen as mutagenic, clastogenic, or genotoxic activity in vivo' (EFSA, 2009b).

44. EFSA (2009a) raised some concern about studies carried out with cinnamaldehyde, that showed it induced chromosomal damage in vitro. However, these findings were not confirmed in in vivo studies. Thus, it was concluded that cinnamaldehyde does not have a genotoxic potential in vivo.

45. A number of Ames tests, sister chromatid exchange, chromosomal aberration and unscheduled DNA synthesis assays as well as transformation assays were also reported by Bickers et al. (2005). Some positive results were cited including sister chromatic exchange assay in Chinese hamster ovary cells, with and without S9 mix; chromosome aberration assay in Chinese CHL hamster fibroblast cells and B241 cells with and without metabolic activation; transformation assay in clones A31-1-13 of mice BALB/c-3T3 cells and Chinese hamster B241 cells; micronucleus assay in human hepatoma Hep G2 cells, male Sprague-Dawley rats and Swiss mice; and DNA fragmentation/alkaline elution assay in male Sprague-Dawley rats. All other studies were negative. The authors concluded that, based on a weight of evidence evaluation of the available mutagenicity and genotoxicity data, as well as metabolism and detoxification, cinnamaldehyde has no significant genotoxic potential under the current conditions of use as a fragrance ingredient.

46. The same assays were cited by WHO and US EPA (US EPA, 2008; WHO, 2001). WHO concluded that 'cinnamyl alcohol and related compounds lack direct mutagenic or genotoxic activity, as indicated by the negative results obtained in bacterial test systems. The mixed results in the assay for DNA repair and in various studies of antimutagenicity were associated with cytotoxicity. Evidence of genotoxic activity was found in isolated mammalian cells, the cinnamyl compounds inducing chromosomal aberrations and/or mutations in the presence or absence of metabolic activation; however, the reported activity in vitro was not seen as mutagenic, clastogenic, or genotoxic activity in vivo' (WHO, 2001). US EPA stated that members of the cinnamyl derivatives category did not show mutagenic potential when tested in vitro in S. typhimurium, apart from a positive test in strain TA100 with cinnamaldehyde. Cinnamaldehyde also induced chromosomal aberrations in vitro, but in vivo data were equivocal (US EPA, 2008).

47. As part of the study described in paragraph 40, Coggins et al. (2011) also carried out an Ames test. In the publication the author stated that, at the highest concentration, cinnamaldehyde showed a significant increase (36 %) in mutagenicity in Salmonella strain TA102 compared to controls. However, in the supplementary data provided with the publication, no statistically significant differences were found between the low, medium and high groups compared with controls.

48. Non-toxic concentrations of cinnamaldehyde increased DNA strand breaks in human pulmonary fibroblasts and human embryonic stem cells, but not lung epithelial A549 cells. DNA damage was reversible in human embryonic stem cells suggestive of efficient repair of DNA once exposure ceases. Authors stated that the data implicate cinnamaldehyde in mutagenicity/genotoxicity and the data were in agreement with other reports that show that cinnamaldehyde induces DNA damage in vitro and in vivo (Behar et al., 2016). Mereto et al. (1994) reported that high doses of cinnamaldehyde increased the frequency of micronucleated hepatocytes but not bone marrow micronucleated polychromatic erythrocytes in rats following a single oral dose (0.5 x LD_{50}) of cinnamaldehyde. Authors concluded that high doses of cinnamaldehyde may induce genetic alterations at the chromosomal level.

Reproductive and developmental toxicity

49. No inhalation route reproductive studies were found for cinnamaldehyde.

Carcinogenicity

50. No inhalation route carcinogenicity studies were found for cinnamaldehyde.

Thermal decomposition of cinnamaldehyde

51. During E(N)NDS use, the vaporisation temperature has been estimated to vary between 40 and 350 °C. The heating period introduces the potential for pyrolysis of compounds. Therefore, thermal degradation and reaction products of flavourings should also be considered (Costigan and Meredith, 2015)[1].

52. 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. 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 ambient flavour[2].

1 This study was carried out by British American Tobacco.
2 This study was carried out by British American Tobacco.

53. Other studies have demonstrated the presence of flavour acetals in E(N)NDS liquids, in the headspace above E(N)NDS liquids and in E(N)NDS vapour (Geiss et al., 2015; Hutzler et al., 2014 and Behar et al., 2018 cited in Erythropel et al. (2018) which suggested that that E(N)NDs liquids may be unstable post-preparation (Erythropel et al., 2018).

54. Cinnamaldehyde propylene glycol acetal activated both TRPA1 and TRPV1 receptors with higher EC50 values compared with cinnamaldehyde. Cinnamaldehyde propylene glycol acetal also activated both receptors at 500 µM, although a dose-response relationship could not be established due to limited solubility (Erythropel et al., 2018).

55. The analytical studies considered by Erythropel et al. (2018) 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). Costigan et al. (2014) estimated that the estimated exposure to cinnamaldehyde propylene glycol acetal from the use of two devices was 47 µg/day[3].

Reviews of toxicity of cinnamaldehyde in E(N)NDS

56. The NAS report (NAS, 2018) based their evaluation of cinnamaldehyde on the in vitro study by Behar et al. (2016), and reported that at non-cytotoxic concentrations, cinnamaldehyde decreased cell growth, attachment, and spreading; altered cell morphology and motility; increased DNA strand breaks; and increased cell death. Overall, the report concluded that ‘in general, studies described above have shown that, even at low concentrations, cinnamaldehyde in e-cigarette products is cytotoxic and genotoxic and adversely affects cell processes and survival. These studies also indicate that cinnamaldehyde in e-cigarettes may impair homeostasis in the respiratory system’.

Summary

57. There are many different flavours of E(N)NDs liquids on the market made up of a number of flavouring chemicals, as well as PG, VG, nicotine and water. Although such flavourings are considered to be GRAS by the US Food and Drug Administration (FDA) or World Health Organization (WHO) via ingestion, few have undergone acute or chronic toxicity testing via the inhalation route. Therefore, the potential toxicity via E(N)NDs use cannot be ascertained. There is, however, some evidence that cinnamaldehyde may be a respiratory irritant following inhalation.

58. The respiratory irritation potential of cinnamaldehyde was investigated. Data from humans indicated that cinnamaldehyde caused upper airway irritation. The RD_{50} was calculated based on physico-chemical parameters as well as in mice. Moreover, cinnamaldehyde activated TRP receptors in vitro. Such receptors are

3 This study was carried out by British American Tobacco.

responsible for eliciting irritation responses in vivo. Overall, data suggest that cinnamaldehyde may act as an airway irritant in E(N)NDs users. Cinnamaldehyde caused skin and eye irritation in animals and humans. Some positive results were obtained in in vitro mutagenicity tests. However, these were not replicated in vivo. Overall, cinnamaldehyde, is not considered to be mutagenic. There were no repeat dose, reproductive or carcinogenicity studies carried out with cinnamaldehyde via the inhalation route.

Questions for the Committee

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

i. Does cinnamaldehyde in e-liquid pose a risk to E(N)NDS users?

ii. Are there any data gaps with respect to the risk assessment for flavouring chemicals 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
May 2019

Abbreviations/Glossary

CC	Conventional Cigarettes
CHO	Chinese Hamster Ovary
Danish EPA	Danish Environmental Protection Agency
E(N)NDS	Electronic Nicotine and Non-Nicotine Delivery Systems
EC_{50}	Concentration required to produce an effect in 50 % of a test population
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC3	Concentration required to provoke a 3-fold increase in lymph node cell proliferative activity compared with controls
EFSA	European Food Safety Authority
ENDS	Electronic Nicotine Delivery Systems
ENNDS	Electronic Non-Nicotine Delivery Systems
EU	European Union
FDA	US Food and Drug Administration
GPMT	Guinea Pig Maximisation Test
GRAS	Generally Regarded As Safe
HRIPT	Human Repeat Insult Patch Test
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
LED	Light-Emitting Diode
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NTP	National Toxicology Program
NZ EPA	New Zealand Environmental Protection Agency
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic Aromatic Hydrocarbons
PG	Propylene Glycol
PII	Primary Irritation Index
QSAR	Quantitative Structural Analysis Relationship
RD_{50}	The concentration required to reduce the mouse respiratory rate by 50 %
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
RIFM	Research Institute for Fragrance Materials
SCCNFP	Scientific Committee On Cosmetic Products And Non-Food Products Intended For Consumers
SCCS	Scientific Committee on Consumer Safety
TRP	Transient Receptor Potential
TSNA	Tobacco-Specific Nitrosamine
US EPA	United States Environment Protection Agency
USA	United States of America
VG	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)

Inhalation toxicity of vanillin

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 ("14371-10-9" OR "104-55-2") OR CHEMNAME (cinnamaldehyde OR "cinnamic aldehyde" OR cinnamaldehyde OR "3-phenylprop-2-enal") OR TITLE-ABS-KEY (cinnamaldehyde OR "cinnamic aldehyde" OR cinnamaldehyde OR "3-phenylprop-2-enal"))) AND ((TITLE-ABS-KEY (*toxic* OR acute OR irritation OR sensitization OR "repeat dose" OR carcin* OR mutagen*) AND TITLE-ABS-KEY (inhal*))): 16

PubMed

(((("14371-10-9"[EC/RN Number] OR "104-55-2"[EC/RN Number]) OR (cinnamaldehyde [Title/Abstract] OR "cinnamic aldehyde" OR "3-phenylprop-2-enal" [Title/Abstract] OR cihinnnamaldehyde[Title/Abstract] OR "3-phenylprop-2-enal"[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]): 7

Search 2: thermal degradation

Scopus

((CASREGNUMBER ("14371-10-9" OR "104-55-2") OR CHEMNAME (cinnamaldehyde OR "cinnamic

aldehyde" OR cinnamaldehyde OR "3-phenylprop-2-enal") OR TITLE-ABS-KEY (cinnamaldehyde OR "cinnamic aldehyde" OR cinnamaldehyde))) AND (TITLE-ABS-KEY ("thermal decomposition" OR "thermal breakdown" OR "thermal degradation" OR thermolysis)): 19

PubMed

(("14371-10-9"[EC/RN Number] OR "104-55-2"[EC/RN Number]) OR (cinnamaldehyde [Title/Abstract] OR "cinnamic aldehyde" OR "3-phenylprop-2-enal" [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.