TOX/2019/49

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

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Follow up to Paper 11: Second draft framework for risk assessment of flavouring compounds in E(N)NDS

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).

2. A number of flavourings are used in E(N)NDS liquids, the toxicity of which has been evaluated via the oral route as many are used to flavour food. However, their toxicity via inhalation has been less well studied and hence few data are available on which to assess the toxicity of flavouring compounds via this route.

3. Two flavouring compounds were considered at the May 2019 COT meeting (TOX/2019/24 and TOX/2019/25), and it was agreed that it would be helpful to develop a decision tree to best utilise the available information for such compounds and focus on potential for toxicity specifically following use in E(N)NDS. The first draft of the decision tree (TOX/2019/37) was discussed at the July 2019 COT meeting. This paper provides a second draft of the decision tree, now called the framework for risk assessment of flavouring compounds, that could be used in considering flavouring compounds intended for inhalation purposes Annex A contains illustrative case studies of use of the framework for the three flavouring substances the Committee has had papers on: vanillin, cinnamaldehyde and menthol. Annex B contains an overview of the relevant CLP classifications.

Question for the Committee

4. Members are invited to comment on the paper, including any aspects that arise from consideration of the case studies, and whether the Committee is content with the amended approach.

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

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Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Follow up to Paper 11: Second draft framework for risk assessment of flavouring compounds in E(N)NDS

Introduction

1. 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').

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

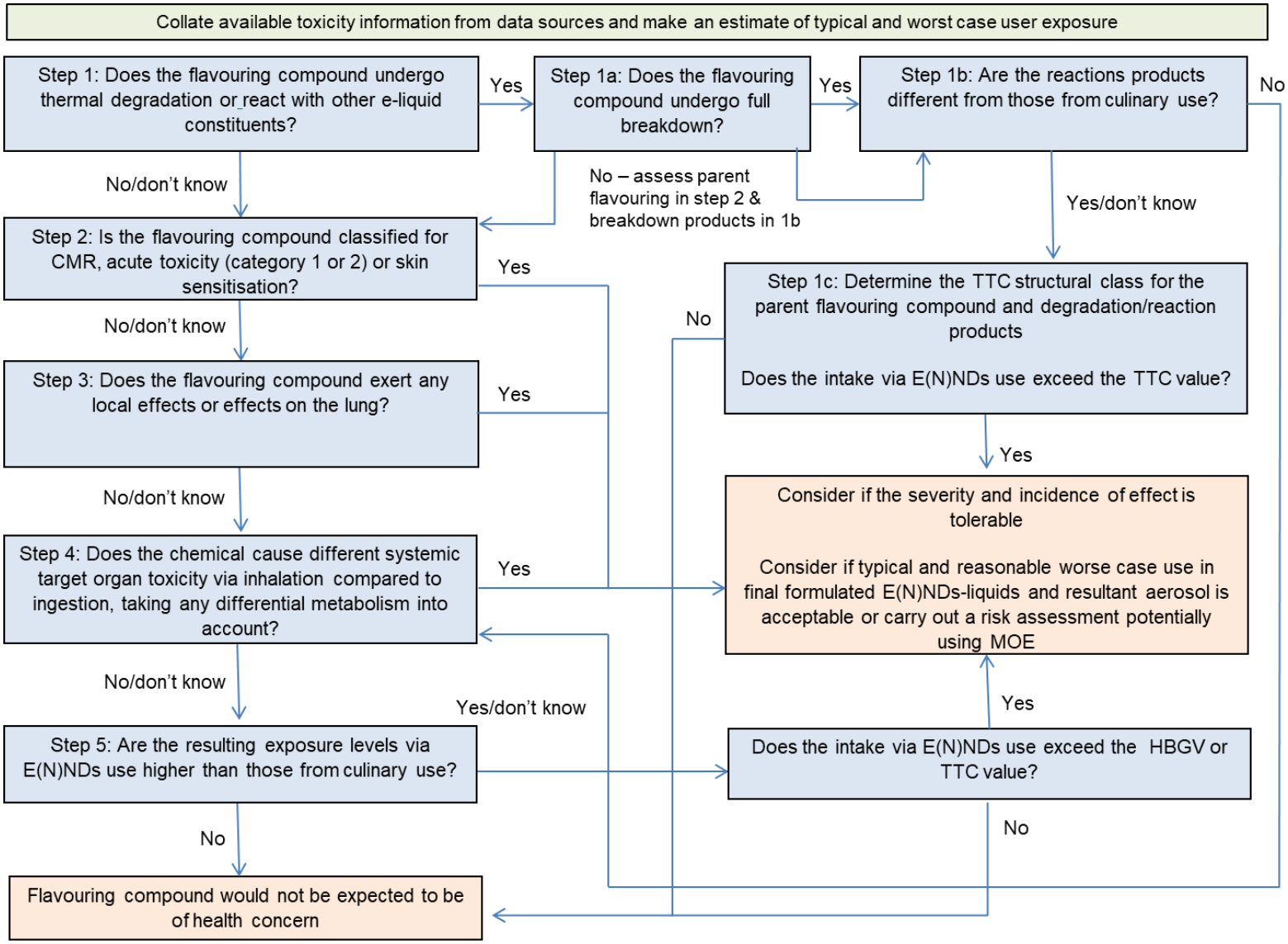
3. Flavouring compounds are one of the five most commonly listed ingredients in E(N)NDS liquids, along with PG, VG, nicotine and water. Over 7000 unique flavours are reportedly available (Erythropel *et al.*, 2018; Zhu and Bonnevie, 2014) although information is not available on the dominant compounds on the UK market.

4. The primary concern about the use of flavouring compounds is that whilst many have been evaluated and approved for use in food, few have undergone acute or chronic toxicity testing via the inhalation route (Fowles and DiBartolomeis, 2017).

Framework for risk assessment of flavouring compounds

5. The framework for risk assessment of flavouring compounds 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.

Figure 1. Framework for risk assessment of flavouring compounds via inhalation exposure



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

Description

6. During E(N)NDS use, the vaporisation temperature has been estimated to vary between 40 and 180 °C. The heating period, influenced by the temperature, length of puff, air flow of the individual puff and how recently the coil was changed introduces the potential for thermal degradation of the compounds. Flavouring compounds may also react with other constituents of e-liquids. Therefore, thermal degradation and reaction products of flavouring compounds should also be considered as part of their risk assessment (Costigan and Meredith, 2015).

Data sources

7. Literature should be searched to identify if thermal degradation products are formed on heating of the flavouring compound and if possible, the concentration formed. Some flavouring compounds, namely, aldehydes and alcohols can undergo chemical reactions with PG, a main constituent of e-liquids, at room temperature to form PG acetals, and ketones for PG ketals (Elmore *et al.*, 2014; Erythropel *et al.*, 2018). Flavouring compounds that form acetals include vanillin, furfural, benzaldehyde, strecker aldehydes, cinnamaldehyde and citral, whilst acetoin, raspberry ketone (4-[4-hydroxyphenyl]-2-butone), and menthone form PG ketals (Elmore *et al.*, 2014).

8. Acetals are sensitive to hydrolysis and may hydrolyse into the parent flavouring compound and PG in the high humidity environment in the respiratory tract or as part of the metabolic pathway (Costigan *et al.*, 2014). Nevertheless, an indication of whether this reaction is likely to occur should be sought in the literature. If new degradation or reaction products are identified, then such products should be assessed using the steps described above.

STEP 1a Does the flavouring compound undergo full breakdown?

Description

9. It is expected that different flavouring compounds with undergo thermal degradation or react with other constituents of e-liquids to different degrees. If 100 % breakdown does not occur then the parent flavouring compound will also need to be assessed for its toxicity as well as breakdown products.

Data sources

10. Literature should be searched to identify if the flavouring compound undergoes full or partial thermal degradation at temperatures similar to those reached by E(ND)NDs. Similarly, if it fully reacts with other constituents of e-liquids or if the parent flavouring compound is still expected to be present.

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

Description

11. Reactions between flavouring compounds and PG are not specific to E(N)NDs liquids. Elmore *et al.* (2014) reported that under acidic or basic conditions, PG can react with food flavourings to give rise to new compounds. Hence, if the use of flavouring compounds in E(N)NDS results in degradation products (thermal or otherwise), information should be sought on whether they are also formed on culinary use of the flavouring. If so, an estimate of systemic exposure by the respective routes should be obtained.

Data sources

12. Literature should be searched to identify if thermal degradation products formed on heating the flavouring compound are different from culinary use.

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?

Description

13. The Threshold of Toxicological Concern (TTC) is a principle that refers to the establishment of a generic exposure level for chemicals, below which the probability that they would cause adverse health effects is low. The TTC approach integrates data on exposure, chemical structure, metabolism, and toxicity consistent with chemical risk assessment principles (EFSA/WHO, 2016). The TTC is intended to provide a health-protective approach in situations where it is not feasible to obtain chemical-specific data, such as impurities and breakdown or reaction products, or where evaluation of a large number of compounds with low exposure is required, such as for flavouring compounds. The TTC approach has been used to evaluate flavouring substances by JECFA, the EC Scientific Committee on Food (SCF) and the European Food Safety Authority (EFSA), albeit via the oral route of exposure (EFSA/WHO, 2016).

14. Carthew *et al.* (2009) and Escher *et al.* (2010) have adapted the oral TTC approach for the inhalation exposure of chemicals. However, due to the limited number of chemicals included in the databases on which the proposed TTC values are based, route-to-route extrapolation from the oral values is considered appropriate. In such cases, bioavailability from the different routes of exposure should be taken into account. As a default, 100 % bioavailability should be assumed (ECHA, 2017a).

Outcome of step 1

15. Flavouring compounds, degradation and/or reaction products that are different to those produced from culinary use, and that do not have sufficient information for application of the framework for risk assessment should be evaluated using the TTC approach with route to route extrapolation from oral values. TTC should be used as part of the weight of evidence assessment of the use of the flavouring in E(N)NDs liquids. Those compounds that exceed their appropriate TTC value should be evaluated for their suitability for use in E(N)NDs liquids. Those that do not exceed the TTC value would not be expected to be of health concern.

16. Flavouring compounds, degradation and/or reaction products that are not different than those produced from culinary use should be assessed with respect to the similarity or difference in the systemic toxicity via oral or inhalation exposure at Step 4 of the framework.

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

Carcinogenicity / Mutagenicity / Reproductive and developmental toxicity

Description

17. In general food flavouring compounds should already be assessed for carcinogenicity, mutagenicity and reproductive and developmental toxicity (CMR) (Costigan *et al.*, 2014). However, as different regions may have different classification criteria some exceptions may exist. Therefore, flavourings under consideration should initially be screened for CMR.

Data sources

- IARC
- Harmonised classification for CMR[1]
- Candidate list of substances of very high concern (SVHCs)
- QSARs
- Self-notified C+L classification for CMR[2]

18. Flavouring compounds that have been classified as being carcinogenic by the International Agency on Research on Cancer (IARC) and their mode of action should be identified. In addition, those that have a Harmonised classification for CMR and

[1] A harmonised classification is a classification for a substance that has been agreed by independent experts at European level, and then made mandatory by law. A harmonised classification is legally binding and suppliers are obliged to use these classifications.
[2] Self-classification is the process through which the supplier classifies the chemicals directly, and where no harmonised classifications are available for the substances involved.

those which have been included on the Candidate list[3] of SVHCs under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations, based on carcinogenicity (Article 57a), mutagenicity (Article 57b) or toxic to reproduction (Article 57c) should be identified. Chemicals that have been self-notified as being CMR should also be listed. Annex B gives an overview of the classification categories.

19. Predictions using QSAR models should also be carried out. Other than DNA-reactivity, predictions should be used as part of an overall weight of evidence approach. Many statistical and mechanistic QSAR models are available to detect mutagenicity, mainly through DNA-reactivity, although fewer models are available for carcinogenicity and reproductive toxicity, due to the complexity of the mechanisms involved. Read across may also be used to predict the CMR potential of the flavouring compounds. Results from the classification and labelling (C+L) notifications may be used in a weight of evidence approach, along with read across predictions and data from QSAR modelling.

Acute toxicity

Description

20. Acute toxicity refers to serious adverse health effects occurring after a single or short-term oral, dermal or inhalation exposure to a substance (ECHA, 2017a; GHS, 2017).

Data sources

- Harmonised classification for acute toxicity
- Self-notified C+L classification for acute toxicity

21. Acute toxicity data such as LC50 values via the inhalation route should be noted.

Skin Sensitisation

Description

22. Skin sensitisation refers to an allergic response following skin contact to a substance. Following a subsequent re-exposure, an immunological mechanism resulting in adverse health effects on the skin (allergic contact dermatitis), can occur (GHS, 2017).

[3] Chemicals that are deemed to be substances of very high concern (SVHCs) based on their hazard are placed on the Candidate list. EU or EEA suppliers of articles which contain substances on the Candidate List in a concentration above 0.1% w/w have to provide sufficient information to allow safe use of the article to their customers.

Data sources

- Harmonised classification for skin sensitisation
- QSARs
- Self-notified C+L classification for skin sensitisation
- Clinical reports and observations

23. Flavouring compounds that have a Harmonised classification for skin sensitisation should be identified. Chemicals that have been self-notified as being a skin sensitiser should be noted. Predictions using QSAR models should also be carried out. Many statistical and mechanistic QSAR models are available to detect skin sensitisation as the steps in the adverse outcome pathway are well understood and serve to describe the applicability domain of a QSAR model or form the basis for grouping substances into chemical categories. Therefore, read across following the Read Across Assessment Framework (RAAF) guidance (ECHA, 2017b), may, also be used to predict the skin sensitisation potential of the flavouring compounds. Results from the C+L notifications may be used in a weight of evidence approach, along with read across predictions and data from QSAR modelling (ECHA, 2017a). Annex B gives an overview of the classification categories.

Outcome of step 2

24. Flavouring compounds that have a harmonised European classification for CMR or acute toxicity (category 1 or 2) or skin sensitisation (category 1) should be evaluated for their suitability for use in E(N)NDs liquids. The severity and incidence of effect should be considered. A risk assessment should be carried out, potentially using a margin of exposure (MOE) approach.

25. Depending on the MOA and other relevant toxicological information, flavouring compounds should also be considered for their suitability in E(N)NDs liquids if they are classed as being carcinogenic by IARC.

26. Compounds should be considered for risk assessment if other data sources described above or other available evidence based on weight of evidence and expert judgement indicate the possibility of the flavouring compounds exerting CMR, acute toxicity or skin sensitisation.

27. If flavouring compounds are not classified as CMR, an acute toxin or a skin sensitiser then local effects on the lung should be considered (step 3).

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

28. A chemical may induce local or systemic effects. A local effect, such as respiratory irritation, is one that is observed at the site of contact, irrespective of whether the chemical is systemically available.

Respiratory irritation

Description

29. The term respiratory irritation is used to indicate two different toxicological effects, namely cytotoxic effects in the respiratory tract and sensory irritation (ECHA, 2017a; GHS, 2017).

30. Cytotoxic effects in the respiratory tract are comparable to dermal and eye irritation, consisting of inflammation (increased blood flow, local infiltration with white blood cells, swelling, oedema), haemorrhage, and eventual necrosis and other pathological changes. Such effects are potentially reversible, depending on the severity, which is dependent on the concentration and duration of exposure. However, chronic irritation can lead to progressive and ultimately irreversible effects such as fibrosis. In addition, the resultant repeated episodes of cell proliferation in the affected tissues, may increase the risk of local tumour development.

31. In contrast, sensory irritation refers to the local interaction of a substance with the autonomic nerve receptors that are widely distributed in the mucosal tissues of the upper respiratory tract. Sensory irritation leads to pain, burning sensation, and tingling, the severity depending on the airborne concentration of the irritant rather than duration of exposure. Sensory irritation is a receptor-mediated effect, and usually occurs almost immediately upon exposure to the inhaled irritant, leading to reflex involuntary responses such as sneezing, lacrimation, rhinorrhea, coughing, vasodilatation of blood vessels in the nasal passages, and changes in the rate and depth of respiration (ECHA, 2017a). It should be noted that sensory irritation is not the same as local irritation (see paragraph 30), and does not progress to any pathological outcome.

32. To date there are no recognised tests for acute respiratory irritation. Acute inhalation studies including histopathological evaluation of the respiratory tract and/or examinations of nasal or bronchioalveolar lavage as well as repeated inhalation studies may provide some information. Substances that cause respiratory tract irritation via a local cytotoxic effect are classified as STOT SE category 3. Those that cause respiratory tract corrosion are classified as STOT SE category 1 or 2, depending on the dose level required to cause the toxicity. Annex B gives an overview of the classification categories.

33. In rodents, sensory irritation leads to a reduction in respiratory rate, which can be determined experimentally by measuring the RD_{50} (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, and is a standard measure of sensory irritation for humans.

34. 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 compounds, such as aldehydes, 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)). *In vitro* tests quantifying the capability of a chemical to activate transient receptor potential (TRP) irritant receptors are currently being considered as replacements for the animal studies to determine the RD_{50}.

35. 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 (ECETOC, 2006). An increased vapour pressure was shown to be correlated with an increased RD_{50} and 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). An ECETOC Task Force, set up to formulate appropriate guidance for data-poor substances, derived a relationship to predict the RD_{50} from the air-water partition coefficient (K_{aw}) and the K_{ow} using the equation below.

$$\text{Log } RD_{50} = b_0 + b_1 \times \log K_{ow} + b_2 \times \log K_{aw}$$

Where:

b_0=6.346; b_1=-0.8333; b_2=0.7139

36. 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.

<u>Data sources</u>

- Harmonised classification
- Self-notified C+L classification
- RD_{50} (*in vivo* data/*in vitro* data/physchem data)
- Clinical reports and observations

37. It should be documented if the flavouring compound has been classified on Specific Target Organ Toxicity following a single exposure (STOT SE) via oral or

inhalation exposure. Annex B gives an overview of the classification categories. If a risk-based approach is applied, potency should be considered as well as the effect.

Respiratory sensitisation

Description

38. Respiratory sensitisation refers to hypersensitivity of the airways after inhalation of a substance (GHS, 2017). Based on the current knowledge, the induction of respiratory sensitisation can occur via inhalation or dermal exposure to the sensitising substance (Redlich, 2010 and Kimber et al., 2015 cited in ECHA (2017a)). Inhalation of e-liquids containing respiratory sensitisers (i.e. type I allergens and causative agents of immediate hypersensitivity) could, over time, lead to IgE-mediated responses, similar to hay fever and occupational asthma (e.g. perennial rhinitis, eczema, breathing difficulties and bronchoconstriction). This may ultimately lead to anaphylactic responses (Costigan *et al.*, 2014).

Data sources

- Harmonised classification for respiratory sensitisation
- Candidate list of SVHCs
- Self-notified C+L classification for respiratory sensitisation
- Clinical reports and observations

39. There are currently no recognised and validated animal or *in vitro* models for testing respiratory hypersensitivity (ECHA, 2017a; GHS, 2017). Annex B gives an overview of the classification categories.

40. Hazard identification and the derivation of tolerable doses are therefore usually based on a weight-of-evidence approach, predominantly from clinical and occupational data, both of which play an important role in identifying any potential hazards. A number of structural alerts for respiratory sensitisation have been identified. Various quantitative structural relationship analysis (QSAR) models contain alerts, including MCASE, Danish (Q)SAR database and Derek Nexus, although they are derived from chemical asthmagens rather than specific respiratory allergens (ECHA, 2017a). The OECD toolbox also contains a profiler (set of rules and structural alerts) for respiratory sensitisation. The profiler helps in grouping substances that share common structural alerts and possibly predicts the respiratory sensitisation potential via read-across (ECHA, 2017a).

41. Flavouring compounds that have been included in the Candidate list of SVHC under REACH, based on respiratory sensitising properties (Article 57(f)) should be identified, and those that have been self-notified as being a respiratory sensitiser should be noted. Identification of structural alerts for respiratory sensitisation using QSAR models and a possible prediction via read-across should be carried out. The RAAF published by ECHA in 2017 can be used as guidance for carrying out read across (ECHA, 2017b).

Effects on the lung

Description

42. Flavouring compounds that have been included in the Candidate list as being a SVHC under REACH based on specific target organ toxicity after repeated exposure (STOT RE), citing the lung as the target organ, (Article 57(f)) and those that have a Harmonised classification should be identified. Annex B gives an overview of the classification categories. Those that have been self-notified as causing STOT RE should be noted.

Data sources

- Harmonised classification
- Candidate list of SVHCs
- Self-notified C+L classification

Outcome of step 3

43. Flavouring compounds that have a harmonised European classification for skin or respiratory sensitisation, respiratory irritation or STOT RE with the lung as a target organ should be considered for their suitability in E(N)NDs liquids. The severity and incidence of effect should be considered. A risk assessment should be carried out, potentially using MOE approach.

44. Depending on the MOA and other relevant information, flavouring compounds should also be considered for their suitability in E(N)NDs liquids if they are on the Candidate list based on respiratory sensitisation.

45. Compounds may also be undesirable based on weight of evidence and expert judgement if other data indicate the possibility of the flavouring compounds exerting respiratory sensitisation effects.

46. For sensitisation effects, it may be possible to identify a threshold below which the risk of sensitisation would be very low using, for example, human no expected sensitisation induction levels (NESILS).

47. If flavouring compounds do not exert local effects on the lung then systemic effects via inhalation and ingestion should be assessed, taking into consideration differential metabolism, under Step 4).

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

Description

48. A chemical may induce local or systemic effects. A systemic effect is one that is observed distant to the site of contact as the chemical becomes systemically available. Secondary effects may occur as a consequence of local effects (ECHA, 2017a; Kuwabara *et al.*, 2007). Local effects are described in paragraph 28.

49. As noted above, many E(N)NDS flavourings are food flavourings, and as such there is information on systemic repeat dose toxicity following oral exposure. However, in general few data are available on the toxicity following inhalation exposure.

Data sources

- Harmonised classification for STOT RE (any organ apart from lung)
- Candidate list of SVHCs
- Self notified C+L classification for STOT RE (any organ apart from lung)
- ADME data
- Evaluations for use as food flavouring
- Clinical reports and observations

50. Flavouring compounds that have been included in the Candidate list as being a SVHC under REACH based on STOT RE (Article 57(f)) and those that have a Harmonised classification should be identified. Annex B gives an overview of the classification categories. Those that have been self-notified as causing STOT RE should be noted.

51. If systemic toxicity is observed via the oral route of exposure, it must be determined if the toxic effects would also occur via inhalation. Kinetic data such as absorption, metabolism, distribution and excretion (ADME) should be collated to assess if the chemical and/or metabolites are likely to reach the systemic circulation following inhalation and oral exposure. It is especially important to understand the metabolism of the flavouring compound as first pass metabolism may occur following ingestion which will not occur following inhalation. In some cases this may result in a reactive metabolite that may not occur following inhalation. Conversely, metabolism may deactivate the flavouring compound hence exposure via inhalation may result in greater systemic toxicity.

52. Information relevant for repeated dose toxicity can also be obtained from data on other endpoints, route-to-route extrapolation from oral studies, structural analogues and physico-chemical properties. Read across may also be used to predict the target organ toxicity via repeated exposure. Results from the C+L

notifications may be used in a weight of evidence approach, along with read across predictions and data from QSAR modelling (ECHA, 2017a).

Outcome of step 4

53. The repeat dose toxicity potential should be used in a weight of evidence judgement with data on the other endpoints.

54. If flavouring compounds exert different toxicity via inhalation compared with ingestion, the severity and incidence of effect should be considered. A risk assessment should be carried out, potentially using a MOE approach.

55. If flavouring compounds exert similar toxicity via inhalation compared with ingestion, then the exposure levels via E(N)NDs use should be considered at Step 5.

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

Description

56. Exposure to flavouring compounds via E(N)NDs use is important to assess the risk. If exposure is similar to or lower than that from culinary use, then the flavouring compound would not be expected to be of health concern.

Data sources

57. Exposure data would need to be gathered including the concentration of the flavouring compound in the aerosol and intake calculations made using generic assumptions regarding E(N)NDs use.

Outcome of step 5

58. If the exposure levels via E(N)NDs use are higher than those from culinary use, then levels should be compared against the TTC value. Those compounds that exceed their appropriate TTC value should be evaluated for their suitability for use in E(N)NDs liquids. Those that do not exceed the TTC value would not be expected to be of health concern.

59. If the exposure levels via E(N)NDs use are similar to or lower than those from culinary use, then the flavouring compound is not expected to be of health concern.

Summary

60. Flavouring compounds are commonly used in E(N)NDS liquids. Despite being approved for use in food, few have undergone acute or chronic toxicity testing via the

inhalation route. Therefore, this framework aims to provide a number of steps designed as a set of principles to guide the risk assessment process for a flavouring compound in E(N)NDS.

61. A number of toxicological endpoints have been included in the framework. Data may be obtained from a number of sources, including evaluations by authoritative bodies such as IARC, EU Harmonised classifications or inclusion on the Candidate list for being an SVHC. Non-animal data may also be used in QSAR modelling and the TTC approach. Using all data available and expert judgement, if the flavouring compound shows the potential to cause any of the endpoints listed, it would be undesirable to include the flavouring compound in E(N)NDs liquids.

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat
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Abbreviations/Glossary

ADME	Absorption, metabolism, distribution and excretion
C+L	Classification and labelling
CC	Conventional cigarette
CEL	Consumer estimated worst case exposure level
CMR	Carcinogenic, mutagenic, reproductive toxin
DST	Dermal sensitisation threshold
E(N)NDS	Electronic Nicotine and Non-Nicotine Delivery Systems
EFSA	European Food Safety Authority
ENDS	Electronic Nicotine Delivery Systems
FDA	US Food and Drug Administration
GRAS	Generally Regarded As Safe
IARC	International Agency on Research on Cancer
IFRA	International Fragrance Association
JECFA	Joint FAW/WHO Expert Committee on Food Additives
K_{aw}	Air-Water Partition Coefficient
K_{ow}	Octanol-Air Partition Coefficient
LED	Light-Emitting Diode
LOAEL	Lowest observed adverse effect levels
MHRA	Medicines and Healthcare Products Regulatory Agency
NESILS	No expected sensitisation induction levels
PAH	Polycyclic Aromatic Hydrocarbon
PG	Propylene Glycol
QSAR	Quantitative structural relationship analysis
RD_{50}	The concentration required to reduce the mouse respiratory rate by 50 %
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
SCF	Scientific Committee on Food
STOT RE	Specific Target Organ Toxicity following a repeated exposure
STOT SE	Specific Target Organ Toxicity following a single exposure
SVHC	Substance of very high concern
TRP	Transient receptor potential
TSNA	Tobacco-Specific Nitrosamine
TTC	Threshold of Toxicological Concern
VG	Vegetable Glycerol

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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). Follow up to Paper 11: Second draft framework for risk assessment of flavouring compounds in E(N)NDS

Case studies of use of framework with vanillin, cinnamaldehyde and menthol

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

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	*ActivatesTRPA1 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.

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

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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	*ActivatesTRPA1 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.

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

References

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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.

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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.

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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 11: Decision tree for risk assessing flavouring compounds in E(N)NDS

The following tables describe the hazard categories for the endpoints discussed in the document according to Guidance to Regulations (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures (https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5).

Tables include:

Table 3.6.1: Hazard categories for carcinogens

Table 3.5.1: Hazard categories for germ cell mutagens

Table 3.7.1 (a): Hazard categories for reproductive toxicants

Table 3.1.1: Acute toxicity hazard categories and acute toxicity estimates (ATE) defining the respective categories

Table 3.4.1. Hazard category and sub-categories for respiratory sensitisers

Table 3.4.2. Hazard category and sub-categories for skin sensitisers

Table 3.8.1. Categories for specific target organ toxicity-single exposure

Table 3.9.1. Categories for specific target organ toxicity-repeated exposure

These tables are provided; for copyright reasons the content of this Annex is not included in the published version on the COT website.

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