Requirements for substances in e-liquids and substances formed during evaporation of e-liquids - elaboration

Study commissioned by:
The Consumer Council at Austrian Standards International and
funded by the Austrian Federal Chambers of Labour

Supervised by:
Dr. Franz Fiala

FORCE Technology
Department of Plastics, Composites and Surface characterisation
Group of Applied Environmental Assessment
Pia Brunn Poulsen
Mirko Miseljic

Final report

11 October 2018

Contents

Summary

Smoking by use of electronic cigarettes (e-cigarettes) is a relatively new phenomenon. In 2012, BfR, the German Federal Institute for Risk Assessment, prepared an opinion on liquids in e-cigarettes and concluded that e-cigarettes cannot be considered safe with respect to health effects. An important risk factor is posed through inhalation of nicotine, however, additional ingredients of the liquids such as solvents, chemical additives, various scent and aroma substances and contaminants can pose health risks (BfR, 2012).

Today, EU legislation exists concerning nicotine containing e-cigarettes (EU Directive no. 40, 2014), which among other things sets requirements for the purity of the ingredients used in e-liquids as well as a requirement of only using ingredients which do not pose a threat to human health. However, requirements for the total content of specific dangerous chemical substances in e-liquids or to the dangerous substances being formed during use (evaporation of e-liquids) are limited – except for a requirement of the level of nicotine in the e-liquids. Standards are now under development in relation to CEN/TC 437 "Electronic cigarettes and e-liquids", which was established in 2015.

During 2017, FORCE Technology carried out two projects for the Austrian Consumer Council at the Austrian Standards Institute (now Austrian Standards International):

- "Requirements for substances in e-liquids used in electronic cigarettes" (Poulsen et al., 2017a)
- "Requirements for substances formed or released during the evaporation of e-liquids used in electronic cigarettes" (Poulsen et al., 2017b)

The purpose of these former two projects was to look more closely at the substances used in e-liquids and the substances formed from e-cigarettes during the evaporation of e-liquids. Based on existing threshold limit values for inhalation of selected substances and a rough calculation of the expected exposure to the ingredients and formed substances, a proposal for limit values for selected ingredients and substances formed was calculated based on risk assessment principles.

The purpose of this study is to elaborate on the two former screening studies in order to refine the results obtained in the former projects. The elaboration includes the following elements:

- Elaboration on threshold limit values for the three main ingredients
- Elaboration on threshold limit values for six selected ingredients or substances formed during the evaporation of e-liquids
- Identification of missing information on use concentrations in the former reports in order to be able to compare the calculated limit values with actual concentrations of use
- Identification of missing levels of quantifications in the articles used in the former reports in order to be able to compare the calculated limit values with actual measured minimum concentrations
- Investigation of irritation effects of selected substances
- Identification of potential respiratory sensitisers

This elaboration project shows the general picture of “limited toxicological data is available” for many of the used ingredients in e-liquids and substances present in e-cigarette vapours. Even though different toxicological threshold limit values are derived by different organisations, the general picture is that the same key study has been used, but different assessment/uncertainty factors have been used thereby resulting in a difference in the derived threshold limit values.

In this elaboration project, no changes to the used threshold limit values for ingredients in e-liquids have been made for the examined main ingredients used in e-liquids (glycerine, propylene glycol and ethylene glycol). The reasons are that no new data seems to be available and that the used threshold limit values in the former project all are derived by reliable organisations.

Minor changes have been made to a few of the calculated limit values for the substances based on occupational threshold limit values. These have been adjusted for continuous exposures (24/8 hours and 7/5 days per week). This results in minor differences in the new calculated limit values (slightly lower limit values).

This elaboration project has also identified use concentrations of some of the ingredients in e-liquids selected in the former project. The total picture of the 15 selected and reviewed ingredients in e-liquids in Poulsen et al. (2017a) is therefore that for 12 of the 15 ingredients the highest use concentration identified in literature or safety data sheets for e-liquids may result in unwanted health effects because the calculated limit value is exceeded. However, for many of the substances – but not the three most often used solvents (glycerine, propylene glycol and ethylene glycol) – use concentrations have also been identified below the calculated limit value. This means that e-liquids are available on the market, which could comply with the calculated limit values (if not considering the three most often used solvents).
Only for one of the 15 reviewed ingredients in e-liquids, the identified used maximum concentration is below the calculated limit value, and the use can be considered as safe concerning long-time inhalation exposure. For the remaining two ingredients, no use concentration was found for comparison.

In the former project that calculated limit values for substances identified in vapours from e-cigarettes (Poulsen et al., 2017b), a total of 13 substances was reviewed. In this elaboration project, the used threshold limit values have been assessed in detail for 6 of these 13 selected substances. In this elaboration project, no changes to the used threshold limit values were suggested for 3 of 6 substances identified in vapour from e-cigarettes, but for the last 3 substances (toluene, xylene and formaldehyde) actually a use of higher threshold limit values was suggested based on the in-depth investigation of toxicological reports available for the substances. This was partly due to use of newer studies available and partly due to a use of lower assessment factors/uncertainty factors. However, the resulting changes in the calculated limit value for these 3 substances do not change the overall conclusion for these 3 substances identified in vapours from e-cigarettes.

Therefore, the overall conclusion for the 13 selected substances identified in vapour from e-cigarettes is still that for 6 of 13 substances use of e-cigarettes may result in unwanted health effects, as the maximum concentrations identified in vapours exceed the calculated limit values for these substances. For the rest of the substances (7 of 13), the identified maximum concentrations in the vapours are all below the calculated limit values. It is, however, important to emphasise that for all 13 substances, there are identified concentrations in vapours from e-cigarettes below the calculated limit values. This means that some e-cigarettes will be able to comply with the calculated limit values.

In this elaboration project, it was also investigated how many of the identified ingredients in e-liquids and substances in vapours from e-cigarettes (from the former two projects) that are classified as irritating for the respiratory tract (H335) or as respiratory sensitisers (H334). Four substances were identified as having a harmonised classification with H335 “May cause respiratory irritation” – all identified in vapour from e-cigarettes. These four substances (acetaldehyde, acrolein, formaldehyde and glyoxal) were all identified with maximum vapour concentrations above the level of which respiratory irritation can occur. This suggests that the maximum concentration levels identified for these four substances (when detected) may result in irritating effects in the throat when inhaled.

Regarding respiratory sensitisation (classification with H334), the search showed that only one substance (cobalt) has a harmonised classification as a respiratory sensitiser (H334 “May cause allergy or asthma symptoms or breathing difficulties if inhaled”). However, in the literature examined in the former projects, cobalt has not been identified in vapour from e-cigarettes and it is therefore not possible to assess whether an actual e-cigarette exposure situation could result in respiratory sensitisation due to inhalation of cobalt.

This elaboration project as well as the two former projects (Poulsen et al., 2017a and 2017b) illustrate that it is necessary to set limit values for frequently used ingredients in e-liquids as well as frequently identified substances in vapours from e-cigarettes, as long-term use of e-cigarettes cannot be considered safe when looking at the maximum identified concentrations in e-liquids and vapours.

1 Introduction

1.1 Background

Smoking by use of electronic cigarettes (e-cigarettes) is a relatively new phenomenon. In 2012, BfR, the German Federal Institute for Risk Assessment, prepared an opinion on liquids in e-cigarettes and concluded that even though smokers of e-cigarettes do not inhale the characteristic carcinogenic combustion products and substances known to be present in tobacco smoke, e-cigarettes cannot be considered safe with respect to health effects. An important risk factor is posed through inhalation of nicotine. Moreover, additional ingredients of the liquids such as fumigation agents (propylene glycol, glycerine), chemical additives, added pharmacologically active compounds, various scent and aroma substances (e.g. menthol, linalool) and contaminants can pose health risks. Furthermore, BfR states that it is difficult to identify the pollutants that contribute to the contamination of indoor air, as the nature of the substances that are inhaled and exhaled often remains unclear (BfR, 2012).

Today, EU legislation exists concerning nicotine containing e-cigarettes (EU Directive no. 40, 2014), which among other things sets requirements for the purity of the ingredients used in e-liquids as well as a requirement of only using ingredients which do not pose a threat to human health. However, requirements for the total content of specific dangerous chemical substances in e-liquids or to the dangerous substances being formed during use (evaporation of e-liquids) are limited – except for a requirement of the level of nicotine in the e-liquids. Standards are now under development in relation to CEN/TC 437 “Electronic cigarettes and e-liquids”, which was established in 2015.

During 2017, FORCE Technology carried out two projects for the Austrian Consumer Council at the Austrian Standards Institute (now Austrian Standards International):

- “Requirements for substances in e-liquids used in electronic cigarettes” (Poulsen et al., 2017a)
- “Requirements for substances formed or released during the evaporation of e-liquids used in electronic cigarettes” (Poulsen et al., 2017b)

The purpose of these two projects was to look more closely at the substances used in e-liquids and the substances formed from e-cigarettes during the evaporation of e-liquids. Based on existing threshold limit values for inhalation of selected substances and a rough calculation of the expected exposure to the ingredients and formed substances, a proposal for limit values for selected ingredients and substances formed was calculated based on risk assessment principles.

1.2 Purpose

The purpose of this study is to elaborate on the two former screening studies carried out on e-liquids for the Consumer Council at Austrian Standards International:

- “Requirements for substances in e-liquids used in electronic cigarettes” (Poulsen et al., 2017a)

- "Requirements for substances formed or released during the evaporation of e-liquids used in electronic cigarettes" (Poulsen et al., 2017b)

The elaboration will encompass elements such as more detailed investigation of the threshold used in the screening projects and other relevant issues to elaborate and refine the results obtained in the two screening projects.

1.3 Definitions

The same definitions as used in the former projects on e-liquids and e-cigarettes carried out for the Consumer Council at Austrian Standards International are used in this report:

According to the Tobacco Directive (EU Directive no. 40, 2014), 'electronic cigarettes' or '**e-cigarettes**', which is the term used in this report, are defined as: "a product that can be used for consumption of nicotine-containing vapour via a mouth piece, or any component of that product, including a cartridge, a tank and a device without cartridge or tank. Electronic cigarettes can be disposable or fillable by means of a refill container and a tank, or rechargeable with single use cartridges".

In this report, the term '**e-liquid**' means any liquid used in e-cigarettes intended for evaporation. The e-liquid may be with or without nicotine. In short, an e-cigarette is a device that is used to transform an e-liquid into an inhalable aerosol. When the e-cigarette user takes a puff on the e-cigarette product, a heating element is activated – and this converts the liquid into an aerosol, which is then taken into the mouth or inhaled, and subsequently exhaled (Colard et al., 2015).

According to the French standard XP D 90-300-2 on electronic cigarettes and e-liquids (Afnor, 2015b), an e-liquid intended for e-cigarettes comprises of the following categories of **ingredients**:

1. A diluent (propylene glycol and/or glycerol and perhaps water)
2. Nicotine (may be added)
3. Flavouring compound (may be added)
4. Other ingredients (may be added)

The diluent currently consists mainly of propylene glycol and glycerol (Afnor, 2015b). However, other diluents such as water or ethanol may be used. A typical composition could be about 80% glycerol and 10 % water, or 20-25% glycerol, about 65% propylene glycol and 5-7% water (Tayyarah & Long, 2014). The maximum content of nicotine allowed is 2% if nicotine is added. However, nicotine-free e-cigarette products are on the market.

E-liquids often contain a high number of different flavouring compounds. Which flavouring compounds that are used differ a lot between the e-liquids. Examples of flavourings are vanillin, menthol and trimethylpyrazine according to the findings in the former reports.

Other ingredients can be either deliberately added ingredients such as the pharma ingredient rimonabant or it can be impurities such as tabacco-specific nitrosamines (TSNA) and different metals.

The **substances formed or released** during the evaporation of e-liquids are defined as substances which are either reaction or degradation products of substances contained in the e-liquid or substances leaching from hardware

components. Some of the substances may also be contained in the e-liquids e.g. impurities. Some metals may for example be present in the liquid (impurities). The vapour resulting from heating the e-liquid is emissions in the form of an aerosol (vapour) consisting of both gas and liquid phase components (BSI, 2015).

1.4 Summary of the former two e-cigarette projects

The former two projects on e-liquids and substances formed from use of e-cigarettes for the Consumer Council at Austrian Standards International were:

1. ”Requirements for substances in e-liquids used in electronic cigarettes” (Poulsen et al., 2017a)
2. “Requirements for substances formed or released during the evaporation of e-liquids used in electronic cigarettes” (Poulsen et al., 2017b)

The two projects both had the purpose to develop a proposal for requirements for selected substances relevant for e-cigarettes. The first project focused on substances in e-liquids and the second project on substances formed or released during the evaporation of e-liquids used in e-cigarettes. The proposal for requirements for selected substances was in both projects based on relevant existing threshold values for the selected substances. Furthermore, the purpose of the studies was to review existing legislation and standards on e-cigarettes, focusing on requirements for e-liquids and substances formed or released during the evaporation of e-liquids.

A review of existing literature listing actual ingredients in e-liquids or substances released from e-cigarettes was carried out. This review resulted in a list of 109 different substances being identified for use in e-liquids and 33 substances being released from evaporation of e-liquids.

The review of the relevant legislation for e-liquids used for e-cigarettes concluded that the Tobacco Directive is relevant for e-liquids and contains a few requirements concerning ingredients in e-liquids and substances formed during the evaporation of e-liquids. However, the actual chemical requirements are limited and are on a more general level. The restrictions for chemical ingredients in e-liquids are limited to:

- A specific limit value for nicotine,
- no use of ingredient such as vitamins, caffeine and similar substances,
- no use of ingredients that colour the emissions from e-cigarettes,
- a general requirement of no use of ingredients that pose a risk to human health (except for nicotine) or are classified as CMR, and
- a list of all ingredients contained in the product, including quantities, must be notified to the competent authorities of the Member States. Furthermore, the toxicological data regarding the ingredients must be notified to the competent authorities of the Member States.

The restriction for chemicals formed or released during the evaporation of e-liquids are limited to:

- Except for nicotine, only ingredients which do not pose a risk to human health in heated or unheated form must be used.
- A list of emissions resulting from the use of the product, including quantities, must be notified to the competent authorities of the Member States. Furthermore, the toxicological data regarding the emissions including when heated must be notified to the competent authorities of the Member States.

The Tobacco Directive, however, only covers e-cigarettes that contain nicotine. Non-nicotine containing e-cigarettes are thereby only covered by the General Product Safety Directive (EU Directive no. 95, 2001) which only uses a general statement saying that products on the market 'must be safe' (including chemical safety).

The review of relevant standards for e-liquids used for e-cigarettes concluded that no standards have been published by CEN/TC 437 "Electronic cigarettes and e-liquids", which was established in 2015. However, in this area a British guidance document exists (BSI, 2015), but it is not to be considered as a standard. Furthermore, a French standard in three parts sets requirements for the e-liquids (part 2) (Afnor, 2015b) and for the emissions from e-cigarettes (part 3) (Afnor, 2016).

The French Afnor (2015b) standard sets specific requirement for the content in e-liquids of 5 specific substances, elemental impurities as well as no use of specific preservatives, sugars, sweeteners, vitamins, radioactive substances etc. The French Afnor (2016) standard sets specific requirements for the release of 5 specific substances including nicotine and the release of 6 specific elements. Furthermore, a general restriction of carcinogenic and potentially toxic substances is given (but no specific limit value or specific substances are listed).
It is therefore concluded in the reports that neither legislation nor existing standards are very specific concerning limit values for dangerous ingredients used in e-liquids or dangerous substances formed or released during the evaporation of e-liquids.

In the two reports, 15 and 13 ingredients/substances formed were selected for which a proposal for limit values of these substances in or formed from evaporation of e-liquids was calculated based on identified existing TLVs (Threshold Limit Values) and based on simple 'worst case' calculations. The 'worst case' calculations were based on an assumption that all of the substance contained in the e-liquid is evaporated, and all of the substance evaporated is inhaled, and finally that all of the inhaled substance is absorbed in the body. It is therefore emphasised in the report that absorption of the substances in the body may therefore be overestimated and the proposed limit values may therefore be lower than necessary.

The worst-case calculations performed in the projects showed that for 9 of the 15 ingredients in e-liquids and for 6 of 13 substances formed or released during evaporation of e-liquids the actual maximum concentrations measured in literature exceed the calculated threshold limit values, i.e. using the e-cigarettes could result in health effects for these substances. The proposed calculated limit values were exceeded by a factor of 10 to 200.

2 Project methodology used

This project is carried out as a desk-top study and includes the following tasks:

1. Elaboration of the results obtained in the two former screening projects
    a. In-depth investigation of thresholds based on recent toxicological data for the main components: glycerine, propylene glycol and ethylene glycol
    b. In-depth investigation of thresholds based on recent toxicological data for 6 selected priority compounds or substances with old or questionable thresholds
    c. Identification of missing LOQs (Level of Quantification) in the previous studies to clarify whether proposed limit values can be met
    d. Investigate irritation effects for selected substances
    e. Identification of potential respiratory sensitisers present in e-liquids or emissions
2. Proposal for new refined requirements for the selected substances present in e-liquids or formed or released during the evaporation of e-liquids
3. Discussion, conclusion and recommendation

3 Elaboration of threshold values

A more thorough investigation has been performed for the threshold limit values for the main components in e-liquids as well as a few other selected components formed or released during the evaporation of e-liquids used in e-cigarettes.

3.1 In-depth investigation of threshold values for main components

A more thorough search has been performed for threshold limit values concerning inhalation for the main components used in e-liquids: glycerine, propylene glycol and ethylene glycol. The result of the search is presented and discussed below.

3.1.1 Glycerine

A search for toxicological information on glycerine provides limited information. The following references were identified:

- US National Library of Medicine TOXNET
- OECD SIDS Initial Assessment Report on glycerol for SIAM, 2002
- CIR (Cosmetic Ingredients Review) Final Report, Safety Assessment of Glycerin as Used in Cosmetics, 2015
- Cosmetic Product Safety Report, 2016 on a cosmetic product containing glycerine. Prepared by DHI, Denmark.

In the former report, the inhalation threshold used in the calculations when assessing the risk of health effects from glycerine when used in e-liquids was 10 mg/m^3, which is the occupational exposure limit value (OECD, 2002). If this value is adjusted for continuous exposure by a factor of 4.2 (from 8 working hours/day to 24 hours/day and 5 working days/week to 7 days/week), the resulting threshold limit value for consumers would be 2.4 mg/m^3.

In TOXNET (2018), the information on inhalation is limited – only an LC_{50} value for inhalation for rats of 570 mg/m^3 for 1 hour is listed[1]. In general, no information concerning threshold limit values for inhalation is listed other than the already identified TLV of 10 mg/m^3. However, in a so-called 'Haz-Map' on glycerine from US National Library of Medicine, a permissible exposure limit (PEL) in the working environment (calculated as an 8-hour total weight average) of 15 mg/m^3 is given for total dust and 5 mg/m^3 for a mist of the respirable fraction[2]. The PEL values are listed by OSHA (Occupational Safety and Health Administration) in the USA.

In the OECD SIDS report on glycerine, it is stated that for inhalation exposure to aerosols of glycerine the NOAEC (No Observed Adverse Effect Concentration) for local irritants effects to the upper respiratory tract is 165 mg/m^3 and 662 mg/m^3 for systemic effects. These values are based on just two studies with animals on glycerine exposure by inhalation. It is also stated that glycerine in general has a low toxicity and this is the reason for the occupational threshold limit value of 10

[1] https://chem.nlm.nih.gov/chemidplus/rn/56-81-5
[2] https://hazmap.nlm.nih.gov/category-details?table=copytblagents&id=501

mg/m^3 (8-hour time weighted average) for glycerine mist, which is used in the working environment in many countries (OECD SIDS, 2002a).

This value of 165 mg/m^3 is the same value as listed in ECHA's database on registered substances for glycerine (or actually the value listed is 167 mg/m^3 to be exact). By using an assessment factor of 5, a DNEL value of 33 mg/m^3 is calculated for the general population for local effects of long term exposure. It is not specified why an assessment factor of 5 is used (ECHA, 2018). This is in contrast to the occupational threshold limit values listed above of 5 and 10 mg/m^3 respectively.

In a cosmetic safety report carried out by DHI in 2016 on a specific cosmetic product containing glycerine, the same inhalation data as listed above is mentioned (DHI, 2016). This could suggest that the toxicological data on glycerine concerning inhalation are limited.

In the CIR safety assessment of glycerine as used in cosmetics (CIR, 2105), the toxicity of incidental inhalation exposure from hair sprays containing either 10% or up to 30% glycerine was discussed. It was concluded that there was little potential for respiratory effects at the reported use concentrations of glycerine because of the low or no toxicity of glycerine (662 mg/m^3) in a 13-week inhalation study (same studies as cited in the references above). It was further noted that 95–99% of droplets/particles would not be respirable to any appreciable amount. This was, however, concluded for the use of glycerine in cosmetic products such as hair sprays where inhalation in most cases will be incidental. For the use of glycerine in e-cigarettes/e-liquid, the situation is different and the exposure will be much more extensive due to higher concentrations (typically 40%), due to the deliberate inhalation of the vapour from the evaporated e-liquid and due to longer exposure time (depending on number of puffs taken per day).

The OECD SIDS (2002a) report on glycerine states that data from studies in humans indicates that glycerine is rapidly absorbed in the stomach. No data on the uptake of glycerine is, however, given concerning inhalation – other than the fact that for inhalation of cigarette smoke containing glycerol, the uptake is listed as 100%, but this value may be listed as worst-case.

All in all, this indicates that no new data concerning toxicity of glycerine by inhalation is available. It is therefore suggested to use the threshold limit value used in the working environment of 10 mg/m^3 and adjusted for continuous exposure, as used in the former report on e-liquids. Alternatively, it may also be justifiable to use the threshold limit value of 5 mg/m^3 (adjusted for continuous exposure) for a mist of the respirable fraction set by OSHA as it is expected that glycerine is vaporised when using the e-cigarettes. Nevertheless, both limit values are in the same order of magnitude and still suggest that the used concentrations in e-liquids today may be too high.

3.1.2 Propylene glycol

A search for toxicological information regarding inhalation for propylene glycol was carried out. The following references were identified:

- ATSDR Toxicological profile for propylene glycol (ATSDR, 1997)
- US National Library of Medicine TOXNET
- OECD SIDS Initial Assessment Report on propylene glycol for SIAM, 2001

- International Journal of Toxicology – Safety Assessment of propylene glycol (Fiume et al., 2012)
- European Medicines Agency (EMA, 2017)
- EPA United States Provisional Peer Reviewed Toxicity Values for propylene glycol (US EPA, 2008)

In the former report, the inhalation threshold value used in the calculations when assessing the risk of health effects from propylene glycol from use of e-liquids was a value of 2.1 mg/m^3. This value was found in the list of 'Agreed EU-LCI values' from December 2016 (EU-LCI, 2016). A new list has been prepared by July 2018, but the EU-LCI threshold value for propylene glycol remains the same.
The EU-LCI background document for propylene glycol can be found in a link to the value in the list of 'Agreed EU-LCI values' from July 2018 (EU-LCI, 2018). According to this background document, this inhalation threshold value of 2.1 mg/m^3 is a derived value which is based on a sub-chronic (90 days) repeated exposure study with rats from 1989 (Suber et al., 1989). In this study, a LOAEC (Lowest Observed Adverse Effect Concentration) of 160 mg/m^3 was established based on a slightly increased incidence in nasal haemorrhaging and ocular discharge. In the background document, it is stated that this study with LOAEC of 160 mg/m^3 is the most relevant dose descriptor for the derivation of a limit value for local effects. In the derivation of a limit value (EU-LCI value), a total assessment factor of 75 is used, which results in an EU-LCI value of 2.133 mg/m^3 or rounded to 2.1 mg/m^3. The total assessment factor of 75 is derived based on an assessment factor (AF) of 10 for intraspecies differences (general population), an AF from LOAEC to NOAEC of 3 and an AF of 2.5 for toxicokinetic differences (standard AF according to ECHA Guidance R8 (ECHA, 2012)).

In ATSDR (1997), it is stated that in general propylene glycol has a low level of toxicity, but there is limited data on health effects following inhalation. ATSDR (1997) also refers to the study by Suber et al. (1989) as a main study on inhalation – the study which the existing EU-LCI value is based on. ATSDR sets a MRL (Minimum Risk Level) for inhalation of 0.009 ppm corresponding to 0.028 mg/m^3 but emphasises that data is insufficient. The MRL set for inhalation for propylene glycol is also based on the study by Suber et al. (1989) and the value of 160 mg/m^3 (or 51 ppm) – however, an assessment factor of 1,000 is used in comparison to the assessment factor of 75 used above for derivation of the EU-LCI value in 2016.

In comparison, the ECHA DNEL value for long-term inhalation and local effects is set at 10 mg/m^3 based on the same LOAEC value of 160 mg/m^3 from Suber et al. (1989) and with a total assessment factor of 15 (5 for intraspecies differences (general population), an AF from LOAEC to NOAEC of 3 and an AF of 1 for interspecies differences as it is stated that "no allometric scaling is needed in case of inhalation exposure or local effects"), (ECHA, 2018). With a LOAEC value of 160 mg/m^3 and a total assessment factor of 15, the calculated DNEL value is 11 mg/m^3, but a value of 10 mg/m^3 is listed due to this value being set for workers' exposure established for aerosols.
According to ECHA (2018), there is limited data on the absorption by inhalation and therefore 100% absorption by inhalation is used.

TOXNET (2018) refers to a working environmental exposures limit[3] of 10 mg/m^3, but otherwise no other specific data on toxicity for inhalation is listed. It is stated that available data is insufficient for derivation of a reference concentration.

[3] https://hazmap.nlm.nih.gov/category-details?table=copytblagents&id=685

In the OECD SIDS (2001) report, no data is given concerning the acute toxicity by inhalation for propylene glycol, and only a few studies concerning long-term toxicity by inhalation are listed. The only long-term study concluding on a NOAEL/LOAEL is the study from Suber et al. (1989) as cited in all the above references.

Fiume et al. (2012) discuss the CIR (Cosmetic Ingredients Review) "Safety Assessment of Propylene glycol as Used in Cosmetics" (from 1994). The CIR Expert Panel concluded that propylene glycol can be used safely in hair sprays because the product size is not respirable and it was concluded that in general propylene glycol can be used safely in concentrations up to 50%.

European Medicines Agency (EMA, 2017) has assessed the use of propylene glycol used as an excipient in medicine. However, in this risk assessment they only conclude on limit values considered to be safe by oral and dermal exposure route. The limit value of 500 mg/kg bw/day for adults does not specifically apply for inhalation.
In this risk assessment of propylene glycol by EMA, it is stated that propylene will be absorbed by the lungs if incorporated in a carrier medium as absorption of vapour is expected to be low due to low vapour pressure. It is also stated that aside from local effects such as nasal haemorrhages, exposure by inhalation to propylene glycol does not pose a significant toxicology problem. These conclusions are, however, based on older data (as listed above) and may not consider the high concentration being inhaled by use of e-cigarettes. No new toxicity data are listed by EMA.

In their "Provisional Peer Reviewed Toxicity Value" document for propylene glycol, US EPA (2008) states that it is not feasible to derive a reference concentration (RfC) for propylene glycol based on the toxicity data for inhalation available for the substance. US EPA also describes the Suber et al. (1989) study, but they conclude that the nasal irritation observed in the Suber study at 160 mg/m^3 (the lowest exposure level used in the study) is most likely a non-specific particulate effect and should not be attributed to propylene glycol itself. This conclusion is also based on the following facts:

- In the Suber study the animals were exposed to an aerosol generated at extreme physical means that would not occur 'naturally' because of the low vapour pressure (0.07 mm Hg at ambient temperature) of propylene glycol.
- Propylene glycol undergoes a rapid photochemical oxidation in air with an estimated half-time of less than one day (ATSDR, 1997).

However, in the case of use of e-liquids with propylene glycol in e-cigarettes, the content of propylene is also generated at extreme physical means (heated in the e-cigarette) and inhaled immediately.

All in all, this indicates that no new data concerning toxicity of propylene glycol by inhalation is available. It is therefore suggested to use the threshold limit value reviewed and discussed for use as an EU-LCI value where they have focused on inhalation. This value of 2.1 mg/m^3 was also used in the former report on e-liquids. From the references discussed above, it is, however, clear that data concerning toxicity of propylene glycol by inhalation is lacking and that the derived threshold limit value is based on the lowest value tested in the study and may be due to physical irritation and not necessarily due to the toxicity of propylene glycol, but no other data on inhalation is available.

3.1.3 Ethylene glycol

A search for toxicological information regarding inhalation for ethylene glycol was carried out. The following references were identified:

- ATSDR Toxicological profile for ethylene glycol (ATSDR, 2010)
- Environment Canada Priority Substances List Assessment Report on ethylene glycol (Environment Canada, 2007)
- Reference Exposure Levels from The Office of Environmental Health Hazard Assessment (OEHHA) in California USA (OEHHA, 2016).
- OECD SIDS Initial Assessment Profile on Ethylene Glycols (Ethylene glycol, Diethylene glycol, Triethylene glycol, Tetraethylene glycol, Pentaethylene glycol) (OECD SIDS, 2004)
- OECD SIDS Dossier on the HPV chemical ethylene glycol (OECD SIDS, 2007)
- WHO Concise International Chemical Assessment Document 45 on ethylene glycol (WHO, 2002).
- US EPA IRIS – Chemical Assessment Summary of ethylene glycol (US EPA, 1987)
- Danish EPA report on evaluation of health hazards by exposure to ethylene glycol and proposal of a health-based quality criterion for ambient air (Nielsen & Ladefoged, 2013)
- US National Library of Medicine TOXNET

In the former report, when assessing the risk of health effects from ethylene glycol from use of e-liquids, the inhalation threshold value used in the calculations was a value of 3.4 mg/m^3. This value was found in the list of 'Agreed EU-LCI values' from December 2016 (EU-LCI, 2016). A new list has been prepared by July 2018, but the EU-LCI threshold value for propylene glycol remains the same.
The EU-LCI background document for ethylene glycol can be found in a link to the value in the list of 'Agreed EU-LCI values' from July 2018 (EU-LCI, 2018). According to this background document, this inhalation threshold value of 3.4 mg/m^3 is a derived value, which is based on a sub-chronic (30 days) repeated inhalation exposure study with humans from 1974 (Wills et al., 1974). In this study, 20 male participants were exposed with respirable ethylene glycol aerosols in a closed room for 20-22 hours/day for 30 days. The concentrations were varied daily, and irritation occurred at 140 mg/m^3 and above. A NOAEC (No Observed Adverse Effect Concentration) for local effects of 67 mg/m^3 was established. The critical effect was irritation of upper respiratory tract. In the background document, it is stated that this study with NOAEC of 67 mg/m^3 is the most relevant study for inhalation exposure. In the derivation of a limit value (EU-LCI value), a total assessment factor of 20 is used, which results in an EU-LCI value of 3.350 mg/m^3 or rounded to 3.4 mg/m^3. The total assessment factor of 20 is derived based on an assessment factor (AF) of 10 for intraspecies differences (general population) and an AF of 2 for other adjustment factors due to high variations in daily tested concentration levels. Furthermore, they point out that no assessment factors are applied or are necessary:

- for study length or exposure duration, since irritation of upper respiratory tract is the critical endpoint,
- for route-to-route or interspecies extrapolations, since the effect is local in the airways and the point of departure is based on inhalation exposure of humans,
- for severity of effect (dose-response) as the critical effect is of low severity.

In comparison, the ECHA DNEL value for long-term inhalation and local effects is set as 7 mg/m^3 based on the same NOAEC value of 67 mg/m^3 from Wills et al. (1974) and with a total assessment factor of 10 (10 for intraspecies differences (general population)), (ECHA, 2018).

In comparison, ATSDR (2010) has established a Minimal Risk Level (MRL) of 2 mg/m^3 for acute-duration inhalation exposure (14 days or less) to ethylene glycol. This MRL is also based on the study from Wills et al. (1974) – however, they state a NOAEL of 23 mg/m^3 and use an uncertainty factor of 10 for human variability and thereby establish the MRL at 2 mg/m^3. No MRL has been derived for chronic-duration inhalation exposure (365 days or more).

Environment Canada (2007) has prepared a Priority Substances List Assessment Report on ethylene glycol and assesses among other things the inhalation risk of ethylene glycol when using consumer products with the substance (e.g. latex wall paints and floor polish). They calculated a tolerable concentration for ethylene glycol of 7.79 mg/m^3 for short-term inhalation exposure based on a nose-only inhalation study with mice (Tyl et al., 1995) with a NOAEL for developmental toxicity of 779 mg/m^3 and a total uncertainty factor of 100 (10 for intraspecies differences and 10 for interspecies differences).

The Office of Environmental Health Hazard Assessment (OEHHA) in California USA has prepared a list of so-called reference exposure levels (REL) for a range of air toxic substances. A REL value for inhalation for ethylene glycol exists as well. It is not stated directly in the list, which toxicity data the REL value is based on, but it is stated that the REL value is based on a chronic study with humans and that the target organs are the respiratory system and the kidney – effects are developmental. The calculated REL for ethylene glycol is 0.4 mg/m^3 (OEHHA, 2016), i.e. a factor of about 10 lower compared to the EU-LCI value, which may be due to the used assessment factors (which are not listed).

In the OECD SIDS background report listing the data used (OECD SIDS, 2007), it is stated that no reliable data is available on acute inhalation toxicity. The study from Wills et al. (1974) is listed but is not further discussed as ‘respiratory tract irritation’ is not a required SIDS element. In the summary conclusions of the assessment of different ethylene glycols, it is stated that ethylene glycols in general are of low toxicity by oral, dermal and inhalation routes. Ethylene glycol is expected to be absorbed 100% when inhaled. Ethylene glycol is reported as causing developmental toxicity in animals, but only by the oral route. Ethylene glycol is recommended as a candidate for further work with a special focus on the developmental toxicity for ethylene glycol. No threshold limit values are listed (OECD SIDS, 2004).

Similarly, in their Concise International Chemical Assessment Document on ethylene glycol, WHO concludes that even though the acute toxicity is low, consistent treatment-related data is lacking for both oral and inhalation exposure. WHO considers the study by Wills et al. (1974) inadequate as a basis for characterisation of exposure-response as it is based on a small number of volunteers and relatively short-term exposure (30 days). For this reason, WHO does not set a threshold limit value for ethylene glycol for inhalation (WHO, 2002).

US EPA IRIS (Integrated Risk Information System) has not established (not evaluated) a reference concentration (RfC) for inhalation of ethylene glycol (US EPA, 1987).

In a report for the Danish EPA, Nielsen & Ladefoged (2013) has established a quality criterion in air (QC_{air}) of 0.012 mg/m^3, i.e. a threshold limit value that is a factor of 283 times lower than the used EU-LCI value. Due to ethical reasons (study on humans), the Danish EPA could not accept the study prepared by Wills et al. (1974). Therefore, the quality criterion in air is based on a study with rats and rabbits by Coon et al. (1970) where a LOAEC of 12 mg/m^3 is established in a sub-chronic 90-day study where the animals were continuously exposed to 12 mg/m^3 ethylene glycol vapours and only this concentration. However, it is noted that this study by Coon et al. (1970) has its limitations as most of the observed effects were interpreted by Coon et al., as being unrelated to the exposure to ethylene glycol. The quality criterion of 0.012 mg/m^3 is calculated by use of a total uncertainty factor of 1,000 (10 for interspecies variations, 10 for intraspecies variability and 10 because a LOAEC is used instead of a NOAEC).

No other relevant data was found in a search in TOXNET (2018).

All in all, this indicates that no new data concerning toxicity of ethylene glycol by inhalation is available. In general, the suggested threshold limit values in the various reports are based on the same study (Wills et al. (1974)) except for limit values set by Environment Canada and the Danish EPA. Generally, the established threshold limit values are in the same order of magnitude (2 to 7.8 mg/m^3) except for the limit value established by California OEHHA of 0.4 mg/m^3 and the Danish EPA of 0.012 mg/m^3. It is, however, not transparent which underlying data is used by OEHHA and the Danish EPA has due to ethical reasons decided to use data from another study even though the observed effect did not seem to be related to ethylene glycol and even though only one concentration level is used in the study. Furthermore, the Danish EPA has used a high uncertainty factor of 1,000.

It is therefore suggested to use the threshold limit value reviewed and discussed for use as an EU-LCI value where they have focused on inhalation. This value also seems to be the most recent established value and it is established by experts. This value of 3.4 mg/m^3 was also used in the former report on e-liquids. It is, however, clear from the references discussed above that data concerning toxicity (and inhalation toxicity) of ethylene glycol is lacking and that the primary reason for the differences in the established threshold limit values is due to the used assessment factors/uncertainty factors. Based on the different established threshold limit values, it could be possible to argue for use of a lower threshold limit value than the EU-LCI-value. Moreover, the derived threshold limit value is based on irritation effects in the upper respiratory tract and not due to inhalation toxicity, but no other data on inhalation seems to be available.

3.2 In-depth investigation of threshold values for selected compounds

In the former two projects, the threshold values that were used for the calculation of the proposed limit values were based on older toxicological data, i.e. data that is more than 5-6 years old or more questionable toxicological data. For some of these substances, a search has been made in order to find more recent and more reliable toxicological data concerning inhalation. The substances selected are:

- Acetaldehyde
- Acrolein
- Glyoxal
- Toluene
- Xylene

The search has only been performed in main documents (assessment reports etc.) found for the substances and does not cover all existing toxicological documents.

Formaldehyde was also chosen as one of the substances where the threshold value should be further investigated, as in 2018, ANSES, the French Agency for Food, Environmental and Occupational Health & Safety, published a new opinion on formaldehyde with new reference values for formaldehyde and thereby new occupational exposure limits (OELs), new toxicity reference values (TRVs) and new indoor air quality guidelines (AIQGs).

3.2.1 Acetaldehyde

The threshold limit value used for acetaldehyde in the former project (Poulsen et al., 2017b) is 0.16 mg/m^3, which is an Indoor Air Quality Guideline (IAQG) value set by ANSES[4], the French Agency for Food, Environmental and Occupational Health & Safety. This threshold limit value is based on a study by Dorman et al. (2008a) where rats in an inhalation study were exposed for 13 weeks. The derived NOAEC was 90 mg/m^3, and the equivalent human NOAEC was calculated as 12 mg/m^3 taking into account the inhalation differences between rats and humans. A total assessment factor of 75 is used (2.5 for variability in toxicodynamics, 10 for interspecies differences, and 3 for adjusting from subchronic to chronic). This results in a threshold limit value (chronic) of 0.16 mg/m^3. The critical effect is listed as degeneration of the olfactory epithelium (olfactory neurones in the nasal cavity) (ANSES, 2014).

In comparison, most other derived threshold values for acetaldehyde, i.e. Environment Canada threshold concentration of 0.39 mg/m^3, US-EPA reference concentration (RfC) of 0.8 mg/m^3 and EU-LCI value of 1.2 mg/m^3 are all based on a study from Appelman et al. (1982 and 1986) (according to EU-LCI background document for acetaldehyde (EU-LCI, 2018). In this inhalation study by Appelman et al. (1982 and 1986), rats were exposed acetaldehyde in a total of 28 days (6 hours per day, 5 days per week). A NOAEC of 270 mg/m^3 was set based on nasal irritation (Environment Canada, 2000a). The difference in the derived threshold limit values is due to the use of different assessment/uncertainty factors.

No data concerning the absorption rate of acetaldehyde has been found.

Therefore, it seems that ANSES is using a newer and lower NOAEC value and the used threshold limit value of 0.16 mg/m^3 therefore seems to be the most correct value to use. However, in this assessment only the year of the studies has been taking into consideration and not the validity of the different studies. In comparison OEHHA (2016) sets a chronic reference exposure level for acetaldehyde at 0.14 mg/m^3. However, it is not possible to see the used toxicological data behind this value.

If the higher EU-LCI value of 1.2 mg/m^3 was to be used instead, this would result in a calculated limit value that would be 13.1 times higher, which would result in a limit value of 33,600 µg/500 puffs instead of 2,560 µg/500 puffs. This would mean that the highest measured value of 6,000 µg/500 puffs in the vapour from e-cigarettes would be below (instead of above) the calculated limit value. This fact implies that it is necessary to evaluate exactly which limit value to set for acetaldehyde. This evaluation should be carried out by toxicological experts.

[4] https://www.anses.fr/en/content/indoor-air-quality-guidelines-iaqgs

It should, however, be noted that the standard by Afnor (2016) also uses the ANSES IAQG value of 0.16 mg/m^3 as the basis of their calculation of a limit value per 200 daily puffs on e-cigarettes.

3.2.2 Acrolein

The threshold limit value used for acrolein in the former project (Poulsen et al., 2017b) is 0.0008 mg/m^3, which is an Indoor Air Quality Guideline (IAQG) value set by ANSES, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, 2013b). The threshold limit value is based on a study by Dorman et al. (2008b) where rats in an inhalation study were exposed for 13 weeks (for 6 hours/day and 5 days/week). The derived NOAEC was 0.5 mg/m^3, and the equivalent human NOAEC was calculated as 0.0667 mg/m^3 taking into account the inhalation differences between rats and humans. A total assessment factor of 75 is used (2.5 for variability in toxicodynamics, 10 for interspecies differences, and 3 for adjusting from subchronic to chronic). This results in a threshold limit value (chronic) of 0.0008 mg/m^3. The critical effect is listed as lesions in the upper respiratory tract (in the epithelium) (ANSES, 2013b).

In comparison, US EPA (2003) sets a reference concentration of 0.00002 mg/m^3 based on an older study (Feron et al., 1978 according to ANSES, 2013b) where the LOAEC was 0.9 mg/m^3 and 0.02 mg/m^3 when adjusted to humans. However, the US EPA uses a total assessment factor of 1,000 compared to 75 by ANSES (2013b).

OEHHA (The Office of Environmental Health Hazard Assessment, California, USA) sets a reference exposure level (REL) for acrolein for inhalation (chronic) at 0.00035 mg/m^3 (OEHHA, 2016). This REL is based on the same study as used in ANSES (2013b), i.e. Dorman et al. (2008b), however, they use an assessment factor of 200 compared to 75 used by ANSES (2013b).

Environment Canada (2000b) derives a tolerable concentration (TC) of 0.0004 mg/m^3 based on a study by Cassee et al. (1996), where a LOAEC of 0.57 mg/m^3 was derived (total assessment factor of 100), (described in ANSES, 2013b).

ATSDR (2007a) derives a minimum risk level (MRL) value for intermediate-duration inhalation (15 to 364 days) of 0.00009 mg/m^3 based on the Feron et al. (1978) study using a LOAEC of 0.4 ppm (0.9 mg/m^3 and 0.02 mg/m^3 when adjusted to humans) and a total assessment factor of 300.

According to ATSDR (2007a), the absorption of acrolein by inhalation is high (> 90% in rats and mice) and was found to be dose- and breathing-rate related.

Therefore, it seems that ANSES uses a newer NOAEC value compared to the other threshold limit values. Furthermore, ANSES uses a NOAEC instead of a LOAEC value, which most of the older threshold limit values are based on. Even though the used ANSES NOAEC value is not the lowest value, it seems to be the correct value to use (newer and a NOAEC instead of a LOAEC). However, in this assessment the validity of the different studies has not been taking into consideration.

In the Afnor (2016) standard, the ANSES IAQG value of 0.0008 mg/m^3 is also used as the basis of their calculation of a limit value per 200 daily puffs on e-cigarettes.

It should be noted that ANSES[5] is currently (2018) reviewing the toxicity reference value (TRV) for acrolein concerning the respiratory route. The resulting indoor air quality guideline (AIQG) from this study should be taken into consideration as a new threshold limit value for the calculation of the limit value in vapour from e-cigarettes.

3.2.3 Glyoxal

The threshold limit value listed for glyoxal in the former project (Poulsen et al., 2017b) is 0.006 mg/m^3, which is for local effects for short-time exposure. Only a few other threshold limit values were identified in the former project – and these were mainly occupational threshold limit values. NICNAS (2014) list an occupational threshold limit value for long-term exposure of 0.1 mg/m^3, which is used in countries like Belgium, Italy, Portugal, Spain, Canada and the USA as an occupational threshold limit value. Other countries use an occupational threshold limit value of 0.5 mg/m^3 (NICNAS, 2014). The threshold limit value used may therefore be a political decided limit value and not necessarily based on a toxicological assessment.
However, in Poulsen et al. (2017b) the lowest limit value found and therefore the limit value used was a tolerable concentration derived based on short-term exposure of 0.006 mg/m^3 by WHO (2004).

In the NICNAS (2014) document, the references used are WHO (2004), OECD SIDS (2003) and SCCP (2005). Common for all these references is that they only describe one inhalation study (reference: Hoechst, 1995) where rats exposed to glyoxal (as an aerosol) for 6 hours/day, 5 days/week for a total of 29 days, resulted in a NOAEC of 0.6 mg/m^3 (0.4 mg/m^3 as nominal concentration) for local effects (in the larynx) and a NOAEC for systemic effects of > 10 mg/m^3 as the maximum used concentration resulted in no systemic effects.
Based on this study, WHO (2004) calculates the tolerable concentration of 0.006 mg/m^3 using a total uncertainty factor of 100 (10 for intraspecies differences and 10 for interspecies differences), which was used in the previous study (Poulsen et al., 2017b) in the calculations.

ECHA (2018) lists a long-term inhalation DNEL value for glyoxal for the general population of 0.44 mg/m^3 and an acute/short-term exposure value of 1.32 mg/m^3 for systemic effects. For local effects by inhalation, the long-term DNEL value for the general population is listed as 0.01 mg/m^3. It should be noted that the DNEL values calculated for inhalation are based on an oral NOAEL value of 25 mg/kg bw/day, which has been converted to a NOAEC – except for the DNEL for local effects, which is based on a NOAEC of 0.4 mg/m^3 (from the study by Hoechst (1995) as listed above).

There is limited qualitative and no quantitative data on the absorption and distribution of glyoxal in humans and experimental animals (WHO, 2004).

Using the Hoechst (1995) study and the standard assessment factors according to ECHA, Guidance R8 (ECHA, 2012) would result in a DNEL value of 0.0005 mg/m^3 for local effects (NOAEC of 0.4 mg/m^3 converted to continuous exposure by a correction factor of 6 hours/18 hours and 5 work days/7 week days) divided by a total assessment factor of 150 (2.5 for toxicokinetic differences, 10 for intraspecies differences and 6 for sub-acute to chronic). This derived threshold limit value is a factor of 12 from the tolerable concentration derived by WHO

[5] https://www.anses.fr/en/content/toxicity-reference-values-trvs

(2004) of 0.006 mg/m^3 and will not change the calculated limit value in Poulsen et al. (2017b) drastically, i.e. the calculated limit value will still be exceeded by the maximum measured concentrations of glyoxal in e-cigarette vapours.

3.2.4 Toluene

The threshold limit value listed for toluene in the former project (Poulsen et al., 2017b) is 0.26 mg/m^3 for long-term exposure effects listed by WHO (2000). This value is an air quality guideline value and is set for potential effects on the developing central nervous system. This value is based on studies of human neurobehavioural effects in the working environment by Foo et al. (1990) and Foo et al. (1993). In these studies, the LOAEC for neurobehavioural effects were approximately 332 mg/m^3. In WHO (2000), this LOAEC is divided by a factor of 4.2 in order to adjust for continuous exposure (24 hours instead of 8 hours work day, and 7 days/week instead of 5 work days/week) and after this a total assessment factor of 300 is used (10 for inter-individual differences, 10 for use of LOAEC instead of NOAEC, and an additional factor of 3 given the potential effects on the developing central nervous system).

Since the WHO (2000) report, an EU-LCI value was established by experts in 2013 by the European LCI Group. In the background document for toluene (can be found in a link in EU-LCI (2018)), several established threshold limit values for different organisations (e.g. US EPA, Canada, RIVM, ATSDR) were evaluated and based on these studies it was decided to use a newer study by Zavalic et al. (1998) (also used by ATSDR (2000)) with a LOAEC of 134 mg/m^3 as the key study. For establishing the EU-LCI value, they used a total assessment factor of 42 to derive the rounded EU-LCI value of 2.9 mg/m^3 (based on an assessment factor of 4.2 for adjustment of exposure duration, a factor of 2 for LOAEC to NOAEC, and a factor of 5 for intraspecies differences (workers to general population)). In comparison ATSDR (2000) calculated a minimum risk level (MRL) value of 0.3 mg/m^3 using a total assessment factor of 4,200.

Since the EU-LCI value established for toluene in 2013, ANSES (2017) and ATSDR (2017) have both established new threshold limit values for chronic inhalation of toluene. ANSES (2017) also uses the above Zavalic et al. (1998) study but describes the value of 123 mg/m^3 as a NOAEC concentration and adjusts the dose over a whole life using a PBPK model to 96 mg/m^3 and afterwards using an interindividual assessment factor of 5 resulting in a chronic inhalation limit value (TRV – toxicity reference value) of 19 mg/m^3.
In contrast, ATSDR (2017) includes other newer studies not listed by ANSES (2017), such as Schäper et al. 2003, 2004, 2008; Seeber et al. 2004, 2005; Zupanic et al. 2002 (cited in ATSDR, 2017), that all state a NOAEC of 45 ppm (about 171 mg/m^3) for neurological effects based on a study of groups of German photogravure printers employed for an average duration of 13.5 years. This NOAEC of 45 ppm was adjusted for continuous exposure (5 days/7 days and 8 hours/24 hours) to 10.7 ppm and was divided by an uncertainty factor of 10 to account for human variability to derive the MRL of 1 ppm (3.8 mg/m^3).

As the used WHO (2000) threshold limit value is based on older data, and as WHO in newer report about indoor air quality guidelines states that the “current evidence for toluene is uncertain or not sufficient for guidelines” (WHO, 2010), it therefore seems appropriate to select another threshold limit value for the bases of the calculation of a limit value in vapour from e-cigarettes. Based on the above listed reports, it is therefore decided to use the newest value set by ATSDR (2017) of 3.8 mg/m^3 instead. The description and calculations in Table 6 and Table 7 have

therefore been adjusted accordingly (corrections have been marked with bold text and blue shading).

Use of a higher threshold limit value will of course result in a higher calculated limit value. The measured values of toluene in e-cigarette vapour were all below the calculated limit value and are therefore also below the new calculated limit value.

3.2.5 Xylene

The threshold limit value listed for xylene in the former project (Poulsen et al., 2017b) is 0.1 mg/m^3 for long-term exposure effects and is listed by US EPA as a reference concentration (RfC) in 2003 and by UBA, the German Environment Agency, as an Indoor Value I (concentration not expected to cause health effects even at lifelong exposure). The RfC value (US EPA) is based on a sub-chronic 3-month study with rats (exposed 6 hours/day and 5 days/week) where a NOAEC of 217 mg/m^3 was established by Korsak et al. (1994). The critical effect in this study was impaired motor coordination in the central nervous system. The NOAEC was adjusted to 39 mg/m^3 considering continuous exposure (24 hours instead of 6 hours, and 7 days/week instead of 5 days/week). A total assessment factor of 300 was used (a factor of 3 for using a sub-chronic study, a factor of 10 for interspecies variability, and a factor of 10 for intraspecies differences).
It has not been possible to identify a background document describing the reasons for setting the UBA Indoor Value I at 0.1 mg/m^3 for xylene. It is, however, stated in (UBA, 2018) that the UBA Indoor Value II for xylenes is 0.8 mg/m^3. Where Indoor Value I represents a concentration of no evidence of lifelong risk, Indoor Value II is an effect-related value based on current toxicological knowledge of the effect threshold of the substance where uncertainty factors have been taken into account.

Since the US EPA established the RfC in 2003, an EU-LCI value was established by experts in the European LCI Group in 2013. In the background document for xylene (can be found in a link in EU-LCI (2018)), several established threshold limit values for different organisations (e.g. US EPA, ATSDR, RIVM, Health Canada, OEHHA and ECHA Registered substances) were evaluated. Based on these studies, it was decided to use a chronic 7-years inhalation study with humans by Uchida et al. (1993), which was also used by ATSDR and OEHHA for deriving threshold limit values. Uchida et al. (1993) established a LOAEC of 62 mg/m^3. For establishing the EU-LCI value, they used a total assessment factor of 126 to derive the rounded EU-LCI value of 0.5 mg/m^3 (based on an assessment factor of 4.2 for adjustment of exposure duration, a factor of 3 for LOAEC to NOAEC, a factor of 5 for intraspecies differences (workers to general population), and a factor of 2 for study length (sub-chronic exposure of 7 years to chronic exposure)). In comparison ATSDR (2007b) used a total assessment factor of 300 for the calculated minimum risk level (MRL) value of 0.175 mg/m^3.

No newer assessments of threshold limit values have been identified, but the assessment by ANSES (2010) was not included in the former report (Poulsen et al., 2017b). However, in ANSES (2010) they do not establish a threshold limit of their own but choose to use the MRL set by ATSDR (2007b) of 0.175 mg/m^3 (or rounded up to 0.2 mg/m^3).

It can therefore be discussed whether the chosen threshold limit value of 0.1 mg/m^3 is the correct threshold to use for the calculation of the limit value in vapour from e-cigarettes. Several organisations, like e.g. EU-LCI and ATSDR, base their

derived threshold limit value on the human study by Uchida et al. (1993) instead of animal studies (the selected threshold value by US EPA), but their choice of safety factors is different. Based on the thorough assessment which has been carried out by experts, it would therefore be possible to argue for a use of the higher threshold value set by EU-LCI of 0.5 mg/m^3 instead. The description and calculations in Table 6 and Table 7 has therefore been adjusted accordingly (corrections have been marked with bold text and blue shading).

Of course, use of a higher threshold limit value results in a higher calculated limit value. The measured values of xylene in e-cigarette vapour were all below the calculated limit value and are therefore also below the new calculated limit value.

3.2.6 Formaldehyde

The threshold limit value listed for formaldehyde in the former project (Poulsen et al., 2017b) is 0.01 mg/m^3, which is a long-term exposure threshold limit value set for indoor air quality (indoor air quality guideline (IAQGs)) listed by (ANSES, 2007; ANSES, 2013a). This threshold limit value is according to the background document (ANSES, 2007) based on a threshold limit value proposed by ATSDR (1999), which is based on a study from Holmstrom et al. (1989), where a LOAEL value of 0.3 mg/m^3 is used and a total assessment factor of 30 (10 for interspecies differences and 3 from LOAEC to NOAEC). The critical effect was histopathological lesions in the nasal region.

ANSES has made an opinion regarding revision of ANSES's reference values for formaldehyde: occupational exposure limits (OELs), derived no-effect levels (DNELs) for professionals, toxicity reference values (TRVs) and indoor air quality guidelines (IAQGs), (ANSES, 2018). In the new ANSES opinion on formaldehyde (ANSES, 2018), it is stated by ANSES that new studies are available and therefore the different threshold limit values for formaldehyde have been revaluated by a group of experts. According to ANSES (2018), new studies reveal that sensory eye irritation is an early effect compared to nasal and respiratory irritation and therefore irritant effects of the eyes were selected as the critical effect for both acute and chronic effects (the most conservative approach as this will also protect against local nasal carcinogenic effects). A study by Lang et al. (2008) conducted with human volunteers resulting in a NOAEC of 0.369 mg/m^3 was selected as the key study when deriving the IAQG for formaldehyde. The total assessment factor used was a factor of 3 for inter-individual differences for the establishment of a chronic threshold limit value. This results in a toxicity reference value of 0.123 mg/m^3, which was then rounded down to 0.1 mg/m^3 for the IAQG. This IAQG was proposed for the protection of the general population for both acute and chronic effects, instead of two different values proposed in ANSES (2007). This new IAQG for formaldehyde is thus a factor of 10 higher compared to the IAQG established in 2007 for long-term effects.

As this re-evaluation of toxicity reference values has been based on new data and has been carried out by experts, it is therefore recommended to use this higher IAQG as the bases for the calculations. Table 6 and Table 7 have therefore been updated accordingly (and are marked with bold text and blue shading). It should be noted that the standard by Afnor (2016) uses the ANSES IAQG value of 0.01 mg/m^3 as the basis of their calculation of a limit value per 200 daily puffs on e-cigarettes.

4 Minor corrections to calculated limit values based on OTLVs

Some of the threshold limit values used in the former reports (Poulsen et al., 2017a and 2017b) were based on OTLVs (occupational threshold limit values). This was true for the substances:

- Glycerine in e-liquids
- Diacetyl in e-liquids
- Acetyl propionyl in e-liquids
- Aluminium found in vapours from e-cigarettes

These values were then in the calculations in the former reports adjusted for 24 hours consumer exposure instead of 8 working hours relevant for occupational threshold limit values. This corresponds to a correction factor of 3. In practise, this correction factor of 3 was used on the 'daily inhalation of air' value of 16 m^3, which resulted in an inhalation value of 5.33 m^3 (16/3).

However, as described in chapter 3 "Elaboration of threshold values", threshold limit values based on occupational exposure are mostly corrected with a factor of 4.2 for continuous exposure (consumer exposure) in the literature. This correction factor of 4.2 accounts for 24 hours of exposure per day instead of 8 hours per working day together with 7 days per week instead of 5 days per working-week (24/8 x 7/5).

For this reason, the OTLVs used for the above substances have been corrected with the same correction factor (of 4.2) to account for continuous (consumer) exposure – and thereby making the foundation for the calculations identical for all substances. However, it should be noted that in the OTLV used for aluminium, a correction factor had already been used to account for continuous exposure.

In practise, the used OTLVs in this report have therefore been adjusted with the correction factor of 4.2 and the value for daily inhalation of air is 16 m^3 in all cases. As a correction factor of 3 was used in the former report (on the 'daily inhalation'-value), this results in slightly lower calculated limit values compared to the limit values calculated in the former two projects. This has been corrected in Table 4 to Table 7 and is marked with bold text and blue shading of the respective cells.

5 Identification of missing information on use concentrations

In the former project on substances identified in e-liquids (Poulsen et al., 2017a), a specific concentration of some substances in the e-liquids were not identified – only the fact that they were used in e-liquids. It has therefore been a task in this study to identify if the concentration of the following substances has been measured in e-liquids:

- Benzophenone
- Coumarin
- Carvone
- Linalool
- Benzyl alcohol

If a concentration is found for these ingredients in e-liquids the proposed limit values can be compared with actual measured concentrations in order to see whether it will be possible to comply with the proposed limit values.

A search was made for the substances listed above in order to identify use levels in e-liquids. Additional information was found for the three flavour substances (fragrances) carvone, linalool and benzyl alcohol and this information is added to Table 5 (new information is marked with light blue background and in bold text), and it can be concluded that all three flavour substances are used in at least one example in concentrations above the calculated limit values.

However, for neither benzophenone nor coumarin it was not possible to find information regarding their concentration of use in e-liquids. This could of course be due to the fact that these two substances are not normally used in e-liquids. Benzophenone and coumarin are discussed in more details below.

5.1 Missing information for benzophenone

In the former project on substances in e-liquids (Poulsen et al., 2017a), benzophenone was identified by Hutzler et al. (2014) in 3 out of 28 e-liquids, but no concentration was listed. In this study, benzophenone was identified by use of chemical analysis (a qualitative screening analysis) of 28 different e-liquids on the market in 2014. The detection limit for identification of benzophenone is not listed, but is assumed to be low, when they have performed a qualitative screening analysis with the purpose of identifying the substances present in the e-liquid and not quantifying the amount.

No information concerning the use concentration of benzophenone in e-liquids was found in the internet search carried out in this elaboration project. This could be due to the fact that benzophenone is used in small concentrations or may be present as an impurity. This is not possible to determine due to no information on concentration levels in Hutzler et al. (2014).

According to the CosIng database (on cosmetic ingredients), benzophenone may be used as an UV absorber in cosmetics. Benzophenone is also used as an UV

absorber in polymers such as PVC, in adhesives, toys, paintings/coatings, food contact materials, and in printing inks (Mikkelsen et al., 2015). Benzophenone may typically be used in concentrations of 0.1% in coatings.

According to REACH registrations, benzophenone may be used in perfume and fragrances, where it may be used with masking purposes, i.e. to reduce or inhibit the basic odour or taste of the product (Mikkelsen et al., 2015).

However, according to the EU Regulation on cosmetic products (EU Regulation 1223/2009), benzophenone is not allowed to be used in cosmetic products as an UV absorber – as it is not listed in Annex VI "List of UV filters allowed in cosmetic products". However, benzophenone may be used for other purposes than UV absorber in cosmetic products. Benzophenone was not identified in the survey they carried out on cosmetic products (Mikkelsen et al., 2015). Its use may be limited because of suspicion of being an endocrine disruptor and because of its restriction in cosmetic products as an UV absorber.

Even though no use concentrations of benzophenone have been identified in e-liquids, both the fact that the calculated proposed limit value is very low 0.73 µg/ml and the fact that the substance is restricted for use as an UV absorber in cosmetic products (products added to the skin), illustrate that the use in e-liquids should be restricted as well.

5.2 Missing information for coumarin

No information concerning the use concentration of coumarin in e-liquids was found in the internet search. This may be due to the fact that coumarin is found naturally in a wide variety of plants and thereby may be found naturally in small amounts in a wide variety of fragrances/flavours. The source, which identified coumarin in 4 out of 28 examined e-liquids (Hutzler et al., 2014), did so by use of chemical analysis (a qualitative screening analysis) of 28 different e-liquids on the market in 2014.

No safety data sheets for e-liquids have been identified where it is noted that coumarin is added deliberately as an ingredient. Hutzler et al. (2014) only describes that coumarin has been identified in 4 of 28 e-liquids, but does not list the concentrations measured. This means that coumarin may have been identified in small amounts suggesting that it is a natural ingredient in other fragrances/flavours.

The calculated limit value of 0.09% (or 878 µg/ml) is low, but may be possible to meet, if coumarin is not deliberately added, but is only present due to its natural occurrence in different fragrances/flavours.

6 Identification of missing LOQs

In the former project on substances formed or released during the evaporation of e-liquids (Poulsen et al., 2017b), some measurements were performed for the substances selected as a focus point in this project. For some of the substances they were only identified in a specific percentage of the analysis performed; in most cases the substances in question were not identified above the level of quantification (LOQ). However, it was not stated in the reference article what the LOQ where. This means it was unknown whether some of the measurements performed actually showed measurements below the proposed limit values. Therefore, the original articles are reviewed in this project in order to see whether the measurements performed illustrate that it will be possible to comply with the proposed limit values. The substances where LOQ levels need to be investigated further are:

- Cadmium
- Chromium (elemental)
- Chromium (III)
- Formaldehyde
- Glyoxal
- Nickel
- Toluene
- Xylene

6.1 New information on LOQ levels

A search was made for the original articles behind the cited articles in Poulsen et al. (2017b). New information regarding LOQ levels has been marked with light blue background and in bold text in Table 7.

6.1.1 Cadmium

For cadmium Cheng (2014) stated that cadmium was found in concentrations from not detected and up to 0.22 µg/150 puffs. However, Cheng (2014) did not cite the detection limit or LOQ from the original article Goniewicz et al. (2014). The original article Goniewicz et al. (2014) was found and this article does not state the detection limit or LOQ either. However, it is stated that in vapours from the 12 e-cigarettes that were tested, cadmium was detected in 11 and only in vapour from one e-cigarette cadmium was not detected. The levels for the vapours from 11 e-cigarettes ranged from 0.01 ± 0.01 µg/150 puffs to 0.22 ± 0.16 µg/150 puffs, corresponding to 0.03 to 0.73 µg per 500 puffs.

According to Farsalinos et al. (2015b), cadmium was detected in 8 of 12 e-cigarettes in concentrations between 0.08 and 1.6 µg/1200 puffs, i.e. between 0.03 and 0.67 µg per 500 puffs. The average concentration was 0.57 µg/1200 puffs corresponding to 0.24 µg per 500 puffs. No LOD or LOQ is listed in the article. It is, however, stated that the original measurements were performed by Goniewicz et al. (2014), but this article does not state the detection limit or LOQ either.

Data from Cheng (2014) and Farsalinos et al. (2015b) therefore originates from the same article. The differences in reported levels may be due to the fact that Farsalinos et al. (2015b) states that they have subtracted background environmental levels before presenting the results or it may simply be rounding of decimals in the calculations for a specific number of puffs. For this reason, the description of the Cheng (2014) study has been deleted from Table 7.

For the calculated proposed limit value of 0.16 µg/500 puffs, this means that actually vapours from 6 of the 12 e-cigarettes would be below the calculated limit value. This information has been updated in Table 7 and the new information is marked with light blue background and in bold text.

6.1.2 Chromium (elemental)

Cheng (2014) cites a study from Laugesen (2008) that chromium is not detected. However, this study from Laugesen (2008) is referring to the e-liquid and not the vapour. In the e-liquid measured, no chromium above 0.2 ppm was detected in an e-liquid. For this reason, this information has been deleted from Table 7.

6.1.3 Chromium (III)

According to Farsalinos et al. (2015b), chromium (III) was found in concentrations up to 0.84 µg/1200 puffs, corresponding to 0.35 µg per 500 puffs. However, Farsalinos et al. (2015b) has reproduced data from measurements from 12 e-cigarettes in Goniewicz et al. (2014) where chromium (III) was not measured and data from measurements of 1 e-cigarette in Williams et al. (2013).

In Williams et al. (2013), a value of 0.007 µg/10 puffs is reported. This corresponds to the 0.84 µg/1200 puffs (equals 0.35 µg per 500 puffs) for the value reported in Farsalinos et al. (2015b). However, in Farsalinos et al. (2015) they state that Williams et al. (2013) does not report whether background environmental levels have been subtracted, i.e. whether the measured value of chromium only originates from the e-cigarette. No LOD nor LOQ is listed in the original Williams et al. (2013) article either. This information has been added to Table 7.

For the one measurement performed for chromium (III), the calculated limit value is therefore above the measured value. However, the number of measurements (1) is very limited.

6.1.4 Formaldehyde

Bekki (2014) cites a study where formaldehyde is found in concentrations in vapours ranging from 0.7 to 140 µg/10 puffs in the 9 of 13 brands, where formaldehyde is detected. However, Bekki (2014) does not list the detection limit, and the detection limit is not found in the originally stated article Uchiyama et al. (2013) either. In the original article it is just stated “not detected”.

New information from the original article is that concentrations of different compounds were measured from a total of 363 e-cigarettes from 13 different brands. Vapours from between 13 and 51 e-cigarettes from each of the 13 brands were measured. Formaldehyde was not detected in vapours from 137 of the 363 e-cigarettes, and according to the original article the concentrations have been measured in mg/m^3 and the measured values for formaldehyde are: 1.3 to 61 mg/m^3

corresponding to 35.8 to 1,678 µg/500 puffs, as a volume of 55 ml was used in this reference for 1 puff. These levels are listed in Table 1 of the article (Uchiyama et al., 2013), however, in the text in the article a maximum value of 260 mg/m^3 (i.e. 7,150 µg/500 puffs) is given for formaldehyde in the vapour from e-cigarettes, but this value cannot be found in the table listing the data.

Because of use of a higher threshold limit value for formaldehyde (see section 3.2.6 "Formaldehyde"), the calculated limit value in the vapour from e-cigarettes is also higher in this report, and the calculated limit value has been adjusted accordingly in Table 7 (and marked with bold text and blue shading).

For the calculated proposed limit value of 1600 µg/500 puffs (or 58 mg/m^3), this means that actually 347 of the 363 vapours from e-cigarettes would be below the calculated limit value. This information has been updated in Table 7 and the new information is marked with light blue background and in bold text.

6.1.5 Glyoxal

Bekki (2014) cites a study where glyoxal is found in concentrations up to 23 µg/10 puffs corresponding to a total amount of 1,150 µg/500 puffs. In all, 13 brands were analysed and glyoxal was detected in 9 of the 13 brands. However, for 12 of 13 brands glyoxal was not detected in any of the specific e-cigarettes tested within each brand. The lowest concentration measured (as listed by Bekki (2014)) is 0.7 µg/10 puffs, corresponding to 35 µg/500 puffs. However, Bekki (2014) does not list the detection limit and the detection limit is not found in the originally stated article Uchiyama et al. (2013) either. In the original article, it is just stated "not detected".

New information from the original article is that concentrations of different compounds were measured from a total of 363 e-cigarettes from 13 different brands. Vapours from between 13 and 51 e-cigarettes from each of the 13 brands were measured. Glyoxal was not detected in vapours from 277 of the 363 e-cigarettes, and according to the original article the concentrations have been measured in mg/m^3 and the measured values for formaldehyde are: 1.3 to 29 mg/m^3 corresponding to 35.8 to 798 µg/500 puffs, as a volume of 55 ml was used in this reference for 1 puff. These levels are listed in Table 1 of the article (Uchiyama et al., 2013), however, in the text in the article a maximum value of 42 mg/m^3 (i.e. 1,155 µg/500 puffs) is given for glyoxal in the vapour from e-cigarettes, but this value cannot be found in the table listing the data.

For the calculated proposed limit value of 96 µg/500 puffs, this means that actually 301 of the 363 vapours from e-cigarettes would be below the calculated limit value. This information has been updated in Table 7 and the new information is marked with light blue background and in bold text.

6.1.6 Nickel

Cheng (2014) cites a study from Laugesen (2008) that nickel is not detected. However, this study from Laugesen (2008) is referring to the e-liquid and not the vapour. In the e-liquid measured no nickel above 0.2 ppm was detected in an e-liquid. For this reason, this information has been deleted from Table 7.

6.1.7 Toluene

Cheng (2014) stated that toluene was found in concentrations from not detected and up to 6.3 µg/150 puffs. However, Cheng (2014) did not cite the detection limit or LOQ from the original article Goniewicz et al. (2014). The original article Goniewicz et al. (2014) was found and this article does not state the detection limit or LOQ either. However, it is stated that in the vapours from the 12 e-cigarettes that were tested, toluene was detected in 10 of the e-cigarettes. The levels for the 10 vapours from e-cigarettes ranged from 0.2 ± 0.0 µg/150 puffs to 6.3 ± 1.5 µg/150 puffs, corresponding to 0.67 to 21 µg per 500 puffs.

For the calculated proposed limit value of 1,387 µg/500 puffs, this means that actually all measured vapour from 12 of the 12 e-cigarettes would be below the new calculated limit value – and actually well below the new calculated limit value. This information has been updated in Table 7 and the new information is marked with light blue background and in bold text.

6.1.8 Xylene

Cheng (2014) stated that xylene was found in concentrations from not detected and up to 0.2 µg/150 puffs. However, Cheng (2014) did not cite the detection limit or LOQ from the original article Goniewicz et al. (2014). The original article Goniewicz et al. (2014) was found and this article does not state the detection limit or LOQ either. However, it is stated that in the vapours from the 12 e-cigarettes that were tested, xylene was detected in 10 and only in two of the e-cigarettes, xylene was not detected. The levels for the 10 vapours from e-cigarettes ranged from 0.1 ± 0.0 µg/150 puffs to 0.2 ± 0.1 µg/150 puffs, corresponding to 0.33 to 0.67 µg per 500 puffs.

For the calculated proposed limit value of 1,600 µg/500 puffs, this means that actually all measured vapour from 12 of the 12 e-cigarettes would be below the calculated limit value – and also well below the calculated limit value. This information has been updated in Table 7 and the new information is marked with light blue background and in bold text.

7 Investigation of irritation effects of selected substances

The purpose of this chapter is to investigate the irritation effects of selected substances used in or released from e-liquids. For this purpose, the classification of the identified substances has been examined. The focus has been on the classification with H335 “May cause respiratory irritation”.

7.1 Substances classified as respiratory irritants

All substances identified in the former two projects on substances used in e-liquids (109 substances) and substances formed or released during the evaporation of e-liquids (33 substances) have been looked up in ECHA’s C&L Inventory. The result of the search is presented in Table 1 below, where only the substances with a classification as H335 is listed. It is, however, only the substances with either a harmonised classification as H335 or substances where more than 50% of the notifiers have notified a classification as H335 that are listed.

Table 1: Substances used in or released from e-liquids with irritation effects (H335: May cause respiratory irritation)

Substance	CAS No.	Present in	Classified as H335
Acetaldehyde	75-07-0	vapour	Harmonised classification
Acrolein	107-02-8	vapour	Harmonised classification
Formaldehyde	50-00-0	vapour	Harmonised classification
Glyoxal	107-22-2	vapour	Harmonised classification
Acetone	67-64-1	vapour	More than 50% of the notifiers have notified H335
Toluene	108-88-3	vapour	More than 50% of the notifiers have notified H335
Xylene	1330-20-7	vapour	More than 50% of the notifiers have notified H335
Acetyl propionyl	600-14-6	e-liquid	More than 50% of the notifiers have notified H335
Benzaldehyde	100-52-7	e-liquid	More than 50% of the notifiers have notified H335
Benzophenone	119-61-9	e-liquid	More than 50% of the notifiers have notified H335
Diacetyl	431-03-8	e-liquid	More than 50% of the notifiers have notified H335
Linalool	78-70-6	e-liquid	More than 50% of the notifiers have notified H335
Menthol	89-78-1	e-liquid	More than 50% of the notifiers have notified H335

When looking at the substances present in vapours from e-cigarettes and with a harmonised classification as H335, these substances are according to the former project on e-cigarettes (Poulsen et al., 2017b) identified in maximum concentrations between 1,150 µg/500 puffs (glyoxal) to 7,150 µg/500 puffs (formaldehyde). In contrast, the three substances present in vapours from e-cigarettes and with no harmonised classification as H335, but with more than 50%

of the notifiers notifying this classification, are identified in concentrations between 0.2 µg/500 puffs (xylene) and 79.8 µg/500 puffs (acetone). The latter three substances are hence identified in much lower concentrations in the vapour from e-cigarettes and are all identified in concentrations below the calculated limit values. Therefore, the focus has been on the four substances identified in the vapour from e-cigarettes with a harmonised classification as H335, and a search for limit values regarding irritation when inhaled has been performed for these substances.

The six substances identified in e-liquids are in the former project (Poulsen et al., 2017a) identified in a maximum concentration between 84 µg/ml (diacetyl) and 130 µg/ml (acetyl propionyl), and with three substances with much higher concentrations of 9,000 µg/ml (0.9%) (linalool), 21,200 µg/ml (2.1%) (benzaldehyde) and 21,600 µg/ml (2.2%) (menthol). However, none of these substances has been identified in the vapour from e-cigarettes (i.e. has not been measured in the identified articles on vapours from e-cigarettes). Therefore, no measurements (concentrations in the vapour) are available for comparison, and consequently the substances are not described further with regard to irritation effects.

7.2 Threshold values for selected respiratory irritants

It is only the main documents concerning toxicity that have been used in the search for threshold limit values concerning respiratory irritation. Therefore, only the main findings are described below.

7.2.1 Acetaldehyde

Only a few data exists concerning respiratory irritation of acetaldehyde. The used NOAEC for the used threshold limit value is based on nasal irritation and not irritation in the throat. However, according to Environment Canada (2000a) throat sensory irritation has been observed following exposure to concentrations just less than 360 mg/m^3 in humans. This value would suggest that the observed maximum concentration of 210 mg/m^3 in the vapour is below the irritation value observed in humans, but not that far below.

ANSES (2014) describes that a value of 3 mg/m^3 in a short-term exposure for 1 hour will result in constriction of bronchi in asthmatics. This value would suggest that asthmatics may experience irritation effects when using e-cigarettes when the acetaldehyde concentrations in the vapours are at its highest (210 mg/m^3).

No inhalation irritation values are described in ECHA (2018) for acetaldehyde.

7.2.2 Acrolein

Only a few data exists concerning respiratory irritation of acrolein. The used NOAEC for the used threshold limit value is based on lesions in the upper respiratory tract, i.e. the nose and not irritation of the throat. However, some human data, mostly from the working environment and of older date, is available. Irritation of nose and throat after just 5 seconds has been found at a concentration of 2.8 mg/m^3 and a significant decrease in respiratory rate after 35 minutes has been found at concentration of 1.4 mg/m^3 (ANSES, 2013b).

ATSDR (2007a) derives a minimum risk level (MRL) for acute-duration inhalation exposure of 14 days or less of 0.003 ppm (0.0069 mg/m^3) based on an old study of acute inhalation exposure of humans, where the LOAEC value of irritation of nose and throat was 0.3 ppm (based on a study by Weber-Tschopp et al. (1977)). They use an uncertainty factor of 100 to derive the acute MRL value.

No inhalation irritation values are described in ECHA (2018) for acrolein.

The above-mentioned irritation levels would suggest that the concentration levels found for acrolein (when detected) of between 1.3 to 73 mg/m^3 may result in irritating effects in the throat when inhaled.

7.2.3 Formaldehyde

ANSES has made an opinion regarding revision of ANSES' reference values for formaldehyde: occupational exposure limits (OELs), derived no-effect levels (DNELs) for professionals, toxicity reference values (TRVs) and indoor air quality guidelines (IAQGs), (ANSES, 2018). In this document, it is stated that eye irritation is the critical effect and occurs before irritation in the throat. In a study of human volunteers by Lang et al. (2008), nasal and respiratory irritation as well as general discomfort was observed at a concentration of 0.369 mg/m^3, but the effect was not always significant. In ATSDR (1999) it is reported that a concentration of 0.5 mg/m^3 to about 3.75 mg/m^3 (3 ppm) can produce symptoms of mild to moderate irritation of the eyes, nose and throat.

A DNEL for local effects (long-term exposure) for the general population is set at 0.1 mg/m^3 (ECHA, 2018). No information is available for short-term exposure.

The above-mentioned irritation levels would suggest that the concentration levels found for formaldehyde (when detected) of between 1.3 to 260 mg/m^3 may result in irritating effects in the throat when inhaled.

7.2.4 Glyoxal

Only a few data exists concerning respiratory irritation of glyoxal. In a study by Hoechst (1995) cited in e.g. OECD SIDS (2003) and SCCP (2005), a NOAEC is given at 0.4 mg/m^3 because of local effects in the larynx at the higher dose of 2.0 mg/m^3. No other effects or irritation effects are described for glyoxal in the investigated references.

No inhalation irritation values are described in ECHA (2018) for glyoxal.

The above-mentioned concentration level of 2.0 mg/m^3 would suggest that the concentration levels for glyoxal (when detected) of 1.3 to 42 mg/m^3 may therefore result in effects in the throat when inhaled (in the highest concentrations of glyoxal measured).

8 Identification of potential respiratory sensitisers present in e-liquids or emissions

The purpose of this chapter is to investigate respiratory sensitisers present in e-liquids or in emissions (vapours) from e-cigarette use. For this purpose, the classification of the identified substances has been examined. The focus has been on the classification with H334 “May cause allergy or asthma symptoms or breathing difficulties if inhaled”.

8.1 Substances classified as respiratory sensitisers

All substances identified in the former two projects on substances used in e-liquids (109 substances) and substances formed or released during the evaporation of e-liquids (33 substances) have been looked up in ECHA’s C&L Inventory. The result of the search is presented in Table 2 and Table 3 below, where only the substances with a classification as H334 are listed.

Table 2: Substances used in e-liquids classified as respiratory sensitisers (H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled)

For substances in e-liquids (11 of 109 substances)		
Substance name	**CAS no.**	**Classification as H334**
Benzoic acid	65-85-0	Yes, notified by 3 of 2158
Chromium (elemental chromium)	7440-47-3	Yes, notified by 298 of 1549
1,8-Cineol	470-82-6	Yes, notified by 2 of 1340
Cobalt	7440-48-4	Yes, harmonised classification
Corylon	80-71-7	Yes, notified by 23 of 1052
Dioctyl phthalate	117-84-0	Yes, notified by 3 of 86
Eugenol	97-53-0	Yes, notified by 8 of 1784
Nickel	7440-02-0	Yes, notified by 1 of 1928
Tadalafil	171596-29-5	Yes, notified by 1 of 66
Thujone	76231-76-0	Yes, notified by 23 of 25
Tin	7440-31-5	Yes, notified by 1 of 578

Table 3: Substances formed from the evaporation of e-liquids classified as respiratory sensitisers (H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled)

For substances formed from the evaporation of e-liquids (5 of 33)		
Substance name	**CAS no.**	**Classification as H334**
Chromium (elemental)	7440-47-3	Yes, notified by 298 of 1549
Formaldehyde	50-00-0	Yes, notified by 2 of 4646
Glyoxal	107-22-2	Yes, notified by 355 of 1051
Nickel	7440-02-0	Yes, notified by 1 of 1928
Tin	7440-31-5	Yes, notified by 1 of 581

Table 2 and Table 3 illustrate that for most of the substances, it is only a small fraction of the substances where many notifiers agree on a classification as respiratory sensitiser (H334). Otherwise, the classification only seems to be used for a very small part of the notifiers (only one or a couple of the notifiers out of e.g.

100 or 1,000 notifiers). The substances relevant to go into more details with on this subject are the only two substances where more than 50% of the notifiers have notified a classification with H334 or where H334 is part of the harmonised classification:

- Cobalt, which has a harmonised classification with H334
- Thujone, where 23 of 25 notifiers have notified a classification with H334

8.2 Properties for selected respiratory sensitisers

In the following section, the above-mentioned two substances will be discussed in more detail with regard to being respiratory sensitisers.

8.2.1 Cobalt

Cobalt was only identified in e-liquids and not in vapour from e-cigarettes – and only by one study (Visser et al., 2015) in the former project Poulsen et al. (2017a). Visser et al. (2015) only detected cobalt in 8 of 183 samples in a maximum concentration of 0.482 µg/ml e-liquid.

Cobalt is as a component of vitamin B_{12} essential in the body (ATSDR, 2004). The toxicological effects of cobalt have been described by e.g. ATSDR (2004) and NIEHS (2002) regarding cobalt dust. Cobalt is a REACH registered substance, but no information is available concerning respiratory sensitisation.

Following inhalation exposure to cobalt-containing particles, the primary target of exposure is the respiratory tract. Primarily respiratory effects, such as asthma, wheezing and decreased lung function, have been reported for occupational exposure of humans to cobalt at levels ranging from 0.015 to 0.13 mg/m^3. Furthermore, animal studies have identified sensitive effects of inhaled cobalt on respiratory tissues. A number of these effects are believed to be the result of the generation of oxidants and free radicals by the cobalt ion. Cobalt exposure also results in sensitisation of the immune system, which may result in asthmatic attacks following inhalation of cobalt in sensitised individuals (ATSDR, 2004). Limited threshold values are given for sensitising effects by inhalation, but it is stated in ATSDR (2004) that the inhalation MRL (Minimum Risk Level) derived at 0.0001 mg/m^3 for chronic-duration inhalation may not be protective for health effects such as hypersensitivity reactions and asthma. A LOAEL of 0.007 mg/m^3 for human sensitisation (occupational exposure) is listed in ATSDR (2004) by Shirakawa et al. (1986, 1988 and 1989) based on occupational exposure (> 3 years) to levels ranging from 0.007 to 0.893 mg/m^3 leading to cobalt sensitisation. The actual minimum exposure level associated with cobalt sensitisation is therefore not known.

No concentration level of cobalt has been identified in the former study Poulsen et al. (2017b) in vapours from e-cigarettes, and therefore it is not possible to assess whether an actual e-cigarette exposure situation could result in respiratory sensitisation due to cobalt.

8.2.2 Thujone

Thujone was only identified in e-liquids and not in vapour from e-cigarettes – and only by one study (Hahn et al., 2014) in the former project Poulsen et al. (2017a).

Hahn et al. (2014) only detected thujone in 4% (2 of 54) of the studied samples. The mean concentration measured was 6.7 mg/l.

The toxicological information on thujone is limited. Thujone is not a REACH registered substance, thus no toxicological information can be found in the ECHA database of registered substances. Thujone is found in essential oils and is naturally present in plants like thyme, sage and rosemary (EMA, 2012).

Cases with severe intoxications in humans have been reported after consumption of essential oils containing thujone. One of the effects is CNS disturbances. Because of thujone being naturally present in some plants an ADI (Acceptable Daily Intake) value of 0.11 mg/kg bw/day has been established for thujone (EMA, 2012). However, in the reports/opinions/assessment identified for thujone, no toxicological information is available concerning inhalation or respiratory sensitisation (EMA, 2012; SCF, 2003; NTP, 2017). For this reason, it is not possible to assess whether the identified concentration of thujone in e-liquids will result in respiratory sensitising effects.

9 Proposal for new refined requirements for the selected substances

In this chapter the revised calculated limit values for the selected substances for either concentrations in the e-liquids (section 8.1) or for concentrations in vapour from e-cigarettes (section 8.2) are presented.

A direct copy of the tables in the two former reports (Poulsen et al., 2017a and 2017b) has been made. The tables from the two former reports have been updated with new or changed threshold limit values. Whenever a change has been made, this has been marked with bold text and blue background colour of the corrected cell.

9.1 Updated proposal for limit values for ingredients in e-liquids

Table 4 and Table 5 below are direct copies of the tables (Table 3 and 4) in the former report (Poulsen et al., 2017a). However, the changes made in this report are marked with bold text and blue background colour of the cell. No changes have been made to the used threshold limit values, but new information has been found regarding used concentrations in the e-liquids.

Table 4: Threshold limit values used in the calculations of limit values for selected substances in e-liquids

Substance	CAS no.	Used TLV (mg/m^3)	Comments	Reference used
Glycerine (glycerol)	56-81-5	**2.38**	Equal to the occupational exposure limit value **(10 mg/m^3 adjusted for continuous exposure by a factor of 4.2)**	OECD, 2002
Propylene glycol (1,2-Propandiol, PG)	57-55-6	2.1	Value set concerning VOC emissions from building products based on detailed assessment of data.	EU-LCI, 2016
Ethylene glycol	107-21-1	3.4	Value set concerning VOC emissions from building products based on detailed assessment of data.	EU-LCI, 2016
Diacetyl (DA)	431-03-8	**0.004**	Occupational threshold limit value (8-hour average, 40 hours, 45 years) based on 1/1,000 excess risk of lung function below normal **(0.01761 mg/m^3 adjusted for continuous exposure by a factor of 4.2)**	NIOSH, 2016
Acetyl propionyl (AP)	600-14-6	**0.009**	Occupational threshold limit value (8-hour average, 40 hours, 45 years) based on 1/1000 excess risk of lung function below normal **(0.03808 mg/m^3 adjusted for continuous exposure by a factor of 4.2)**	NIOSH, 2016
Lead	7439-92-1	0.0005	Annual average concentration in air	WHO, 2000
Cadmium	7440-43-9	0.000005	Level set in air to prevent any further increase of Cd in agricultural soil	WHO, 2000

Substance	CAS no.	Used TLV (mg/m^3)	Comments	Reference used
Nickel	7440-02-0	0.0000025	Based on a risk of 1/1,000,000 of cancer by inhalation	WHO, 2000
Benzophenone	119-61-9	0.000152	Initial risk screening level for air	Michigan, 2015
Coumarin	91-64-5	0.183	DNEL for consumers based on long term inhalation	ECHA Registered Substances Database
Carvone	6485-40-1	0.289	DNEL for consumers based on long term inhalation	ECHA Registered Substances Database
Linalool	78-70-6	0.7	DNEL for consumers based on long term inhalation	ECHA Registered Substances Database
Benzaldehyde	100-52-7	0.02	German Indoor Air, Guide Value I: conc. in indoor air not expected to cause adverse health effects in sensitive persons in case of lifelong exposure	UBA, 2016
Benzyl alcohol	100-51-6	0.4	German Indoor Air, Guide Value I: conc. in indoor air not expected to cause adverse health effects in sensitive persons in case of lifelong exposure. Similar value by EU-LCI (2016)	UBA, 2016 (EU-LCI, 2016)
Menthol	89-78-1	16.3	DNEL for consumers based on long term inhalation	ECHA Registered Substances Database

Table 5: Proposal for limit values for 15 selected ingredients in e-liquids.
Limit values marked with light red background are exceeded by existing levels found in e-liquids.
Limit values marked with light green background are not exceeded by existing levels found in this review in e-liquids.

Substance	CAS no.	Values used	Limit value µg/mL e-liquid (500 puffs)	Limit value µg/mL e-liquid (200 puffs)	Comments / comparison with AFNOR standard value or actual measurements
Glycerine (glycerol)	56-81-5	V_{air_daily} = **16 m³** $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = **2,38 mg/m³**	**11,429** **(11 mg/mL or 1.1%)**	**28,571** **(29 mg/mL or 2.9%)**	Found in concentrations from 7 to 42% (solvent) **in 28 of 28 e-liquids**. Average content was 26% (Hutzler et al., 2014). A content of 42% equals 420,000 µg/mL assuming a density of 1.
Propylene glycol (1,2-Propandiol, PG)	57-55-6	V_{air_daily} = 16 m³ (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 2.1 mg/m³	10,080 (10 mg/mL or 1.0%)	25,200 (25 mg/mL or 2.5%)	Found in concentrations from 2 to 79% (solvent) **in 28 of 28 e-liquids**. Average content was 53% (Hutzler et al., 2014). A content of 79% equals 790,000 µg/mL assuming a density of 1.
Ethylene glycol	107-21-1	V_{air_daily} = 16 m³ (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 3.4 mg/m³	16,320 (16.3 mg/mL or 1.6%)	40,800 (40.8 mg/mL or 4.1%)	Found in concentrations from 1 to 76% (solvent) **in 13 of 28 e-liquids**. Average content was 26% (Hutzler et al., 2014). A content of 76% equals 760,000 µg/mL assuming a density of 1. Is restricted by the Afnor (2015b) standard by no use as ingredient.
Diacetyl (DA)	431-03-8	V_{air_daily} = **16 m³** $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = **0.004 mg/m³**	**20**	**50**	Found in concentrations from 26 to 278 µg/day (Farsalinos et al., 2015a), i.e. 8 to 84 µg/mL assuming use of 3.3 mL/day (equals 500 puffs). Is restricted by the Afnor standard by no use as ingredient. Afnor (2015b) standard target value: 22 mg/L, i.e. 22 µg/mL Approved for food use, but associated with respiratory disease when inhaled (Shraufnagel, 2014).
Acetyl propionyl (AP)	600-14-6	V_{air_daily} = **16 m³** $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = **0.009 mg/m³**	**44**	**109**	Found in concentrations from 20 to 432 µg/day (Farsalinos et al., 2015a), i.e. 6 to 130 µg/mL assuming use of 3.3 mL/day (equals 500 puffs). Approved for food use but associated with respiratory disease when inhaled.
Lead	7439-92-1	V_{air_daily} = 16 m³ (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.0005 mg/m³	2.4	6	Found in concentrations up to 4.93 µg/ml. However, only 16 of 183 samples above LOQ (5 ng/ml). Afnor (2015b) standard target value: 10 mg/L, i.e. 10 µg/mL
Cadmium	7440-43-9	V_{air_daily} = 16 m³ (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.000005 mg/m³	0.024	0.06	Found in concentrations up to 81 ng/ml, i.e. 0.081 µg/mL. However, only 6 of 183 samples above LOQ (1 ng/ml) Afnor (2015b) standard target value: 1 mg/L, i.e. 1 µg/mL

Substance	CAS no.	Values used	Limit value µg/mL e-liquid (500 puffs)	Limit value µg/mL e-liquid (200 puffs)	Comments / comparison with AFNOR standard value or actual measurements
Nickel	7440-02-0	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.0000025 mg/m^3	0.012	0.03	Found in concentrations up to 225.9 µg/mL. However, only 27 of 183 samples above LOQ (10 ng/mL equalling 0.010 µg/mL).
Benzophenone	119-61-9	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.000152 mg/m^3	0.73	1.8	*No indication on concentration levels found in this review.* In 3 of 28 e-liquids (Hutzler et al., 2014), but no concentrations listed.
Coumarin	91-64-5	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.183 mg/m^3	878 (0.09%)	2,196 (0.2%)	*No indication on concentration levels found in this review* In 4 of 28 e-liquids (Hutzler et al., 2014), but no concentrations listed. Prohibited in tobacco in Germany (Hutzler et al., 2014)
Carvone	6485-40-1	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.289 mg/m^3	1,387 (0.1%)	3,468 (0.3%)	**Found in the following concentrations in SDSs: > 1 ≤ 5%; ≥ 0,5 ≤ 0,75%.** In 2 of 28 e-liquids (Hutzler et al., 2014).
Linalool	78-70-6	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.7 mg/m^3	3,360 (0.3%)	8,400 (0.8%)	**Found in the following concentrations in SDSs: 0.1-0.9%; < 0.5%. 0.9% corresponds to 9,000 µg/mL.** In 6 out of 28 liquids (Hutzler et al., 2014); **In 5 of 50 e-liquids (Kurcharska, 2016); In 5 of 29 e-liquids (Nieuwesigaret, 2018).** Common ingredient in perfumes, where it is regulated by European Cosmetics Directive.
Benzaldehyde	100-52-7	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.02 mg/m^3	96	240	Found in concentrations up to 21,200 µg/mL, i.e. 2.1% (Hutzler et al., 2014). However, only identified in 4 out of 28 e-liquids examined.
Benzyl alcohol	100-51-6	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.4 mg/m^3	1,920 (0.2%)	4,800 (0.5%)	**Found in the following concentrations in SDSs: 1-1.5%; ≥ 1% and < 10%; 2-12%; ≤ 2.5%.** In 3 out of 28 liquids (Hutzler et al., 2014); **In 18 of 50 e-liquids (Kurcharska, 2016); In 6 of 29 e-liquids (Nieuwesigaret, 2018).** Regulated by European Cosmetics Directive.

Substance	CAS no.	Values used	Limit value µg/mL e-liquid (500 puffs)	Limit value µg/mL e-liquid (200 puffs)	Comments / comparison with AFNOR standard value or actual measurements
Menthol	89-78-1	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 16.3 mg/m^3	78,240 (78.2 mg/mL or 7.8%)	195,600 (195.6 mg/mL or 19.6%)	Found in concentrations up to 21,600 µg/mL (i.e. 2.2%) (Hutzler et al., 2014). However, only identified in 12 out of 28 e-liquids examined.

9.2 Updated proposal for limit values for substances formed during the evaporation of e-liquids

Table 6 and Table 7 below are direct copies of the tables (Table 3 and 4) in the former report (Poulsen et al., 2017b). However, the changes made in this report are marked with bold text and blue background colour of the cell.

Changes have been made to the used threshold limit values for formaldehyde, toluene and xylene, and updated information has been added for glyoxal. Of course, this results in other calculated limit values for these three substances. Finally, new data regarding the concentration identified in vapours from e-cigarettes or missing LOD/LOQ levels has been added (or deleted) for a range of substances. This has all been marked with bold text and blue shaded background of the respective cell in the tables.

Table 6 Threshold limit values identified and used in the calculations of limit values for selected substances formed during the evaporation of e-liquids

Substance	CAS no.	Lowest identified TLV (mg/m^3)	Comments
Acetaldehyde (ethanal)	75-07-0	Short-term exposure: 3.0 mg/m^3 (ANSES, 2013a) Long-term exposure: 0.160 mg/m^3 (ANSES, 2013a)	No information in ECHA (2017). ANSES (2013a) lists IAQG for short-term exposure (1 h) at 3.0 mg/m^3 (irritation of airways, bronchoconstriction) and long-term exposure (> 1 y) at 0.160 mg/m^3 (damage of epithelium, possibly carcinogenic). US EPA IRIS (1991) lists a RfC of 0.009 mg/m^3 (degeneration of olfactory epithelium (long-term effect)). They use conservative safety factors, which are not supported by current ECHA guidelines. For this reason, this value is not used even though it is the lowest value. EU-LCI (2013) is set at 1.2 mg/m^3.
Acetone	67-64-1	Short-term exposure: 511 mg/m^3 (ACC, 2003) Long-term exposure: 200 mg/m^3 (ECHA, 2017)	American Chemistry Council (ACC, 2003) lists a RfC of 29 ppm (74.2 mg/m^3) based on a NOAEL of 2200 ppm for developmental toxicity by inhalation. A value for short term exposure is set at 200 ppm (511 mg/m^3), which is NOAEL for throat irritation. ECHA (2017) lists a limit value for consumers of 200 mg/m^3 based on long term inhalation. An overall assessment factor of 2 is used with the EU OEL of 1210 mg/ m^3 as the basis, which has been corrected for 24 hours exposure instead of eight hours working exposure. ATSDR MRL(1994) sets a limit of 13 ppm (33.2 mg/m^3) for chronic exposure and 26 ppm for acute exposure based on neurological effects. An uncertainty factor of 100 is used. AgBB (2015) sets a limit of 1.2 mg/m^3, which, however, is the German or EU OEL of 1210 mg/m^3 divided by a safety factor of 1000. For long-term exposure for the general population, the ECHA DNEL value of 200 mg/m3 is used, as the other values seem of older date and use conservative safety factors, which are not supported by current ECHA guidelines.
Acrolein (propenal)	107-02-8	Short-term exposure: 0.0069 mg/m^3 (ANSES, 2013b) Long-term exposure: 0.0008 mg/m^3 (ANSES, 2013b)	US EPA (2003) sets a RfC of 0.02 $\mu g/m^3$ (sub-chronic effects in nose). ATSDR MRL (2007) for inhalation is 0.00004 ppm (0.0987 $\mu g/m^3$). ANSES (2013b) sets a short-term limit value (1 hour) at 3 ppb or 6.9 $\mu g/m^3$ and a long-term (> 1 year) at 0.35 ppb or 0.8 $\mu g/m^3$. No information for consumer inhalation in ECHA (2017). However, DNEL value for long-term inhalation for workers is 0.2 mg/m^3. The ANSES long-term value is used in the calculations even though it is not the lowest value (US EPA). The ANSES value is, however, the most recent value.
Aluminium	7429-90-5	Short-term exposure: 50 mg/m^3 (Krewski et al., 2007) Long-term exposure: 0.0055 mg/m^3 (Michigan DEQ, 2015)	In a risk assessment, Krewski et al. (2007) has determined the relevant exposure level of concern for irritation following inhalation to be 50 mg/m^3. Long-term exposure for inhalation not described. Michigan DEQ (2015) has set a RfC of 0.0055 mg/m^3 based on human data of a long-term occupational exposure with a critical effect of psychomotor and cognitive impairment. **This value has been corrected for continuous exposure by a factor of 2.8 (Michigan DEQ, 2015).** No information for consumer inhalation in ECHA (2017). However, DNEL value for long-term inhalation for workers is 3.72 mg/m^3.

Substance	CAS no.	Lowest identified TLV (mg/m^3)	Comments
Cadmium	7440-43-9	Short-term exposure: 0.00003 mg/m^3 (ATSDR, 2012) Long-term exposure: 0.00001 mg/m^3 (ATSDR, 2012)	ATSDR (2012) MRL for inhalation is 0.03 $\mu g/m^3$ (acute, short-term exposure – an uncertainty factor of 300 is used) and 0.01 $\mu g/m^3$ (chronic, long-term exposure – an uncertainty factor of 9 is used). US EPA IRIS has not listed a RfC for inhalation for cadmium. ECHA (2017) lists a DNEL value for workers for long-term exposure of 4 $\mu g/m^3$. WHO (2000) sets an air quality guideline at 5 ng/m^3 in order to prevent any further increase of Cd in agricultural soils likely to increase the dietary intake of future generations. The values from ATSDR are used for the calculations as they are from recent years and because the WHO air quality guideline is set low in order to prevent accumulation of Cd in soils.
Chromium (elemental)	7440-47-3	Short-term exposure: *none for elemental Cr* Long-term exposure: 0.027 mg/m^3 (ECHA,2017) 3 µg/day (EMA, 2016)	ECHA (2017) lists a DNEL value for consumers for long-term exposure of 0.027 mg/m^3. WHO (2000) air quality guideline is 0.025 ng/m^3, based on a risk of 1/1,000,000 of cancer by inhalation. However, this value is for Cr (VI) and not chromium itself. US RAIS (1992a) lists a nasal irritation inhalation value of 0.01 mg/m^3, and a long-term inhalation limit value of 0.012 $\mu g/m^3$ for carcinogenic effects – but again this limit value is for Cr (VI). US EPA (1998) has not established a RfC for elemental chromium, only for chromium VI, which is 0.000008 mg/m^3 based on respiratory effects in humans. Based on risk assessments, EMA (2016) has defined PDE (Permitted Daily Exposure) values for elemental impurities in drugs. PDE for inhalation of elemental chromium is 3 µg/day, which can be used as the *safe daily total amount*.
Chromium (III)		Intermediate-term exposure: 0.0001 mg/m^3 Long-term exposure: *none*	ATSDR (2012) MRL for inhalation of soluble chromium (III) is 0.1 $\mu g/m^3$ for intermediate exposure – an uncertainty factor of 300 is used. US EPA (1998) has not established a RfC for chromium (III) (insoluble salts) as data are considered to be inadequate.
Formaldehyde	50-00-0	Short-term exposure: **0.1 mg/m^3** (ANSES, **2018**) Long-term exposure: **0.1 mg/m^3** (ANSES, **2018**)	**ANSES (2018) lists one IAQG for both short-term and long-term exposure of 0.1 mg/m^3**. Critical effect is **eye** irritation. The limit value is also low enough to protect against local nasal carcinogenic effects. ECHA (2017) lists a DNEL value of 0.1 mg/m^3 for the general population (inhalation route) for local effects (long term exposure). Most critical effect in humans seems to be sensory irritation of the eyes (OECD, 2002) with a threshold of 0.1 ppm (Golden, 2011) or 0.08 ppm (Naya & Nakanishi, 2005). However, effect may not be relevant for inhalation of e-liquid. Golden (2011) lists a TLV of 0.1 ppm (0.13 mg/m^3) for nasal cancer as a result of inhalation.

Substance	CAS no.	Lowest identified TLV (mg/m^3)	Comments
Glyoxal	107-22-2	Short-term exposure: 0.006 mg/m^3 (WHO, 2004) Long-term exposure: 0.1 mg/m^3 (NICNAS, 2014)	NICNAS (2014) has listed different occupational TLVs for glyoxal around the world. Lowest TLV for short-term exposure is 0.3 mg/m^3 and 0.1 mg/m^3 for long-term exposure. WHO (2004) has used a NOEL value for local effects in the larynx of 0.6 mg/m^3 to establish a tolerable concentration limit value of 0.006 mg/m^3 for local effects for short-time exposure. ECHA **(2018)** has listed the following DNEL values for consumers for inhalation: local effects **and long-term exposure** (0.01 mg/m^3); **systemic effects** and long-term exposure (1.3 mg/m^3); **systemic effects and long-term exposure (0.44 mg/m^3)**.
Lead	7439-92-1	Short-term exposure: *None* Long-term exposure: 0.0005 mg/m^3 (WHO, 2000)	US EPA (2004) has not evaluated a RfC for lead based on the available data. WHO (2000) air quality guideline for lead is 0.5 $\mu g/m^3$, which is an annual average lead level in air that should not be exceeded (i.e. long-term exposure).
Nickel	7440-02-0	Short-term exposure: 0.8 mg/m^3 (ECHA, 2017) Long-term exposure: 0.0000025 mg/m^3 (WHO, 2000)	ECHA (2017) has listed the following DNEL values for consumers for inhalation: long-term exposure (0.020 mg/m^3), local effects (0.8 mg/m^3). WHO (2000) calculates a value of 0.0000025 mg/m3 (or 2.5 ng/m3) associated with a cancer risk of 1/1,000,000 inhalation.
Toluene	108-88-3	Short-term exposure: **7.6 mg/m^3** (**ATSDR, 2017**) Long-term exposure: **3.8 mg/m^3** (**ATSDR,2017**)	ECHA (2017) has listed the following DNEL values for consumers for inhalation: short-term exposure (226 mg/m^3) and long-term exposure (56.5 mg/m^3), local effects. WHO (2000) sets a limit value of 0.26 mg/m^3 as guideline value for potential effects on the developing central nervous system (long term effects), **but newer data indicates that other higher data is more relevant**. **ATSDR (2017) lists a MRL value of 7.6 mg/m^3 for acute-duration (14 days or less) for inhalation exposure, and a MRL value of 3.8 mg/m^3 for chronic-duration (365 days or more) for inhalation exposure.** **ANSES (2017) lists a TRV of 19 mg/m^3 for chronic exposure and 21 mg/m^3 for acute exposure.**
Xylene	1330-20-7	Short-term exposure: 174 mg/m^3 (ECHA, 2017) Long-term exposure: **0.5 mg/m^3 (EU-LCI)**	ECHA (2017) has listed the following DNEL values for consumers for inhalation: inhalation short-term exposure (174 mg/m^3), local effects (260 mg/m^3), long-term exposure (14.8 mg/m^3). German AgBB and EU-LCI list the limit value of 0.5 mg/m^3. ATSDR **(2007b)** lists a MRL value of 0.05 ppm corresponding to **about 175 mg/m^3**. US EPA RfC and German Indoor Value I is 0.1 mg/m^3. **German Indoor Value II is 0.8 mg/m^3 (based on toxicological threshold effects).** NICNAS (2013) lists different occupational exposure limits in different countries between 25-100 ppm (108-435 mg/m^3). Critical effect is neurobehavioural effects.

Table 7: Proposal for limit values for 12 selected substances which are formed during the evaporation of e-liquids
Limit values marked with light red background are exceeded by existing maximum levels found evaporating from e-liquids.
Limit values marked with light green background are not exceeded by existing maximum levels found in this review evaporation from e-liquids.

Substance	**CAS no.**	**Values used**	**Limit value µg/500 puffs (equal to safe dose)**	**Limit value µg/m³ (based on 500 puffs)**	**Comments / comparison with AFNOR standard value or actual measurements**
Acetaldehyde	75-07-0	V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 0.160 mg/m³	2,560	93,091	Found in concentrations up to 8 µg/puff (Hutzler et al., 2014) and in concentrations up to 120 µg/10 puffs (Bekki, 2014) **or 210 mg/m³ (Uchiyama et al., 2014)**, corresponding to a total of 6,000 µg for 500 puffs. In Bekki (2014), 13 brands of e-cigarettes were analysed, where acetaldehyde was detected in 9 of 13 brands. Lowest concentration measured (if detected) was 0.2 µg/10 puffs, i.e. 10 µg/500 puffs. No LOD or LOQ is listed. In comparison Afnor (2016) calculates a total acceptable amount of 3200 µg (and relates this amount to 200 puffs).
Acetone	67-64-1	V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 200 mg/m³	3,200,000	116,363,636	Present in most vapours (Varlet, 2015). Found in concentrations between 0.16 and 2.9 mg/m³ or 2,900 µg/m³ (Cheng, 2014) **– corresponding to a maximum concentration of 79.8 µg for 500 puffs**. No information regarding how often acetone is identified from evaporation of e-liquids.
Acrolein	107-02-8	V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 0.0008 mg/m³	12.8	465.5	Found in concentrations up to 3 µg/puff (Hutzler et al., 2014) and in concentrations up to 40 µg/10 puffs (Bekki, 2014) **or 33 µg/10 puffs (73 mg/m³) according to Uchiyama et al. (2014)**. This corresponds to a total of **1,650 to** 2,000 µg for 500 puffs. In Bekki (2014), 13 brands of e-cigarettes were analysed, where acrolein was detected in 9 of 13 brands. Lowest concentration measured (if detected) was 0.6 µg/10 puffs, i.e. 30 µg/500 puffs **(or 1,3 mg/m³)**. No LOD or LOQ is listed. BfR (2012) found acrolein in concentrations up to 9.3 mg/m³. In comparison Afnor (2016) calculates a total acceptable amount of 16 µg (and relates this amount to 200 puffs).

Substance	**CAS no.**	**Values used**	**Limit value µg/500 puffs** **(equal to safe dose)**	**Limit value µg/m³** **(based on 500 puffs)**	**Comments / comparison with AFNOR standard value or actual measurements**
Aluminium	7429-90-5	V_{air_daily} = **16 m³** (TLV**adj**. 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 0.0055 mg/m³	**88**	**3,200**	A 'real-life' study showed a 2-fold increase of aluminium in indoor air after vaping (Pisinger, 2014). No actual level stated. According to Saffari (2014) aluminium is not detected in emissions. Measured in 1 e-cigarette in a concentration of 47.28 µg/1200 puffs corresponding to 19.7 µg/500 puffs (Farsalinos, 2015b).
Cadmium	7440-43-9	V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 0.00001 mg/m³	0.16	6	Farsalinos (2015b) detected cadmium from 8 of 12 e-cigarettes. Concentrations were between 0.08 and 1.6 µg/1200 puffs, **corresponding to between 0.03** and up to 0.67 µg per 500 puffs. No LOD or LOQ is listed, **nor in the original article (Goniewicz et al. (2014)**. **Half (6 of 12) measurements are found to be below the calculated limit value, and the rest above the calculated limit value.**
Chromium (elemental)	7440-47-3	V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 3 µg/day	3	109	Found in concentrations of 0.7 ng/puff, corresponding to a total of 0.350 µg (per 500 puffs)), (Cheng, 2014). Saffari (2014) states that higher concentrations are measured compared to normal cigarettes (28.1 ng/h). In comparison Afnor (2016) calculates a total acceptable amount of 3 µg (and relates this amount to 200 puffs) – based on EMA (2016).
Chromium (III)		V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 0.0001 mg/m³	1.6	58	Found in concentrations of **0.007 µg/10 puffs (Cr(III))**, equal to 0.35 µg/500 puffs **(Williams et al., 2013).** **However, Cr(III) was only measured in vapours from 1 e-cigarette. No LOD or LOQ is listed**.

Substance	**CAS no.**	**Values used**	**Limit value µg/500 puffs** **(equal to safe dose)**	**Limit value µg/m³** **(based on 500 puffs)**	**Comments / comparison with AFNOR standard value or actual measurements**
Formaldehyde	50-00-0	V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = **0.1 mg/m³**	**1600**	**58,182**	Found in concentrations up to 5 µg/puff (Hutzler et al., 2014) and up to 140 µg/10 puffs (Bekki, 2014) **or 260 mg/m³ (Uchiyama et al., 2014)** corresponding to a total maximum amount of **7,150 µg for 500 puffs.** In Bekki (2014), a **total of 363 e-cigarettes from 13 different brands** of e-cigarettes were analysed, where formaldehyde was detected in 9 of 13 brands **(or in 226 of 363 e-cigarettes)**. Lowest concentration measured (if detected) was **1.3 mg/m³**, i.e. 35.8 µg/500 puffs. No LOD or LOQ is listed. BfR (2012) found formaldehyde in concentrations up to 8.3 mg/m³ (8,300 µg/m³). In comparison Afnor (2016) calculates a total acceptable amount of 200 µg (and relates this amount to 200 puffs).
Glyoxal	107-22-2	V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 0.006 mg/m³	96	3,491	Found in concentrations up to 23 µg/10 puffs **or 42 mg/m³ (Uchiyama et al., 2014)** corresponding to a total amount of 1,150 µg for 500 puffs (Bekki, 2014). In Bekki (2014), **a total of 363 e-cigarettes from** 13 **different** brands of e-cigarettes were analysed, where glyoxal was detected in 9 of 13 brands **(or in 86 of 363 e-cigarettes)**. In 12 out of 13 brands glyoxal was not detected for some of the tests performed for the specific e-cigarettes **within each brand**. Lowest concentration measured (if detected) was **1.3 mg/m³,** i.e. 35 µg/500 puffs. No LOD or LOQ is listed.
Lead	7439-92-1	V_{air_daily} = 16 m³ (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 0.0005 mg/m³	8	291	Found in concentrations from 0.8 to 4.4 µg/1200 puffs with an average of 0.7 µg/1200 puffs and was detected in all 13 e-cigarettes examined (Farsalinos, 2015b). These values correspond to between 0.33 to 1.8 µg/500 puffs with an average of 0.3 µg/500 puffs.

Substance	**CAS no.**	**Values used**	**Limit value µg/500 puffs** **(equal to safe dose)**	**Limit value µg/m³** **(based on 500 puffs)**	**Comments / comparison with AFNOR standard value or actual measurements**
Nickel	7440-02-0	V_{air_daily} = 16 m^3 (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = 0.0000025 mg/m^3	0.04	1.5	Found in concentrations between 2 and 7 ng/10 puffs (Cheng, 2014) corresponding to between 0.1 and 0.35 µg for 500 puffs. According to Saffari (2014), nickel is measured in higher concentrations compared to normal cigarettes (130.5 ng/h). In comparison Afnor (2016) calculates a total acceptable amount of 5 µg (and relates this amount to 200 puffs) – based on EMA (2016).
Toluene	108-88-3	V_{air_daily} = **16 (TLV 24h)** V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = **3.8 mg/m³**	**60,800**	**2,210,909**	Found in concentrations between not detected **(in vapour from 2 of 12 e-cigarettes)** and **between 0.2 and** up to 6.3 µg/150 puffs **in vapour from 10 of 12 e-cigarettes measured** (Cheng, 2014). Found in concentrations up to 0.63 µg/15 puffs (Drummond, 2014) corresponding to a total amount of 21 µg for 500 puffs. No LOD or LOQ is listed.
Xylene	1330-20-7	V_{air_daily} = 16 m^3 (TLV 24h) V_{per_puff} = 0.055 L n_{puffs} = 500 puffs Used TLV = **0.5 mg/m³**	**8,000**	**290,909**	Found in concentrations between not detected **(in vapour from 2 of 12 e-cigarettes)** and **between 0.1 and** up to 0.2 µg/150 puffs (Cheng, 2014) corresponding to a total amount of **0.3 to** 0.7 µg for 500 puffs. No LOD or LOQ is listed.

10 Discussion and recommendations

In the former two projects carried out for Austrian Standards International concerning substances identified for use in e-liquids (Poulsen et al., 2017a) and substances identified in vapours from e-cigarettes (Poulsen et al., 2017b), limit values (in e-liquids and in vapour from e-liquids) were calculated based on worst-case exposure situations and based on identified existing and often the lowest threshold limit values from literature.

In this project, some of the used existing threshold limit values from literature have been discussed and the 'correctness' of the used threshold limit values has been evaluated based on insights into the used study behind the threshold limit values and the used assessment/uncertainty factors. Furthermore, a search for more data on the actual used concentrations of the substances identified in e-liquids has been carried out in order to be able to compare the calculated limit values with actual used concentrations.

This elaboration project shows a general picture for many of the used ingredients in e-liquids and substances present in e-cigarette vapours: limited toxicological data is available. Even though different toxicological threshold limit values are derived by different organisations (US EPA, EU-LCI, AgBB, ATSDR, OEHHA, Environment Canada, ECHA etc.), the general picture is that the same key study has been used, but different assessment/uncertainty factors have been used thereby resulting in a difference in the derived threshold limit values. Of course, the latest derived threshold values may have used newer key studies instead with more valid or relevant data.

In this elaboration project, no changes to the used threshold limit values for ingredients in e-liquids have been made for the examined main ingredients used in e-liquids (glycerine, propylene glycol and ethylene glycol). The reasons are that no new data seems to be available and that the used threshold limit values in the former project all are derived by reliable organisations. It may be possible to argue for use of other threshold limit values, but the difference and the resulting modification of the calculated limit value would not change the fact that these three substances, which are solvents, all are used in high concentrations – much higher concentrations than the calculated limit values. The use of these solvents should therefore be discussed by toxicological experts and limit values should be established for their use in e-liquids.

It should, however, be noted that for propylene glycol, the derived threshold limit value is based on a study where the lowest value tested is used as a LOAEC, and where it is stated that the effect observed may be due to physical irritation and not necessarily due to the toxicity of propylene glycol, but no other data on inhalation is available. Similar for ethylene glycol, the derived threshold limit value is based on irritation effects in the upper respiratory tract and not due to inhalation toxicity, but no other data on inhalation seems to be available.

Minor changes have been made to a few of the calculated limit values for the substances based on occupational threshold limit values. These have been adjusted for continuous exposures (24/8 hours and 7/5 days per week). This results in minor differences in the new calculated limit values (slightly lower).

This elaboration project has also identified use concentrations of some of the ingredients in e-liquids selected in the former project (Poulsen et al., 2017a). The identified use concentrations illustrate that also the ingredients carvone, linalool and benzyl alcohol are used in higher concentrations than the calculated limit values. This suggests that a use of e-liquids with these ingredients may result in unwanted health effects. The total picture of the 15 selected and reviewed ingredients in e-liquids in Poulsen et al. (2017a) is therefore that for 12 of the 15 ingredients the highest use concentration identified in literature or safety data sheets for e-liquids may result in unwanted health effects because the calculated limit value is exceeded. However, for many of the substances – but not the three most often used solvents (glycerine, propylene glycol and ethylene glycol) – use concentrations have also been identified below the calculated limit value. This means that e-liquids are available on the market, which could comply with the calculated limit values (if not considering the three most often used solvents). For two of the selected 15 ingredients (benzophenone and coumarin), no use concentrations were identified. This could be due to the fact that these two substances are used in very low concentrations and perhaps are only found as impurities in different flavour ingredients in e-liquids. Only for one of the 15 selected ingredients (menthol), the identified used maximum concentration is below the calculated limit value. The use of menthol in e-liquids can therefore be considered as safe concerning long-term inhalation exposure which has been considered in the calculations.

In the former project which calculated limit values for substances identified in vapours from e-cigarettes (Poulsen et al., 2017b), a total of 13 substances was reviewed. In this elaboration project, the used threshold limit values have been assessed in detail for 6 of these 13 selected substances. Furthermore, a search was made to identify the lowest reported concentrations in vapours for 8 of the 13 substances, i.e. information on missing LOQ (level of quantification) was investigated. This new information was added to the overview tables and the tables were updated.

Regarding the assessment of the used threshold limit values, no change was suggested in the used threshold limit values for 3 of the substances, but for the last 3 substances (toluene, xylene and formaldehyde) actually a use of higher threshold limit values was suggested based on the in-depth investigation of toxicological reports available for the substances. This was partly due to use of newer studies available and partly due to a use of lower assessment factors/uncertainty factors. The resulting changes in the calculated limit value for these 3 substances do, however, not change the overall conclusion for these 3 substances identified in vapours from e-cigarettes. For formaldehyde, the highest identified concentration in the vapour is still above the calculated limit value, but it is obvious that more e-cigarettes will be able to comply with the higher calculated limit value. For both xylene and toluene, the highest identified concentrations in the vapour were below the calculated limit value before and are of course also below the now higher calculated limit value.

Therefore, the overall conclusion for the 13 selected substances identified in vapour from e-cigarettes is still that for 6 of 13 substances use of e-cigarettes may result in unwanted health effects, as the maximum concentrations identified in vapours exceed the calculated limit values for these substances. For the rest of the substances (7 of 13), the identified maximum concentrations in the vapours are all below the calculated limit values. It is, however, important to emphasise that for all 13 substances, concentrations in vapours from e-cigarettes below the calculated limit values are identified. This means that some e-cigarettes will be able to comply with the calculated limit values.

It should be noted that for all substances, except for glyoxal, the calculated limit values are based TLVs for long-term effects. The calculated limit value for glyoxal is based on a local short-term TLV (the lowest identified TLV).

In this elaboration project, it was also investigated how many of the identified ingredients in e-liquids and substances in vapours from e-cigarettes (from the former two projects) that are classified as irritating for the respiratory tract (H335) or as respiratory sensitisers (H334). Four substances were identified as having a harmonised classification with H335 "May cause respiratory irritation" – all identified in vapour from e-cigarettes. Nine other substances had no harmonised classification with H335, but more than 50% of the notifiers had notified a classification as H335. Of these, three substances were identified in vapour from e-cigarettes and six substances only as ingredients in e-liquids. The four substances with a harmonised classification with H335 (acetaldehyde, acrolein, formaldehyde and glyoxal) were all identified with maximum vapour concentrations above the level of which respiratory irritation can occur. This suggests that the maximum concentration levels identified for these four substances (when detected) may result in irritating effects in the throat when inhaled.

Regarding respiratory sensitisation (classification with H334), the search showed that only one substance (cobalt) has a harmonised classification as a respiratory sensitiser (H334 "May cause allergy or asthma symptoms or breathing difficulties if inhaled"). However, in the literature examined in the former projects, cobalt has not been identified in vapour from e-cigarettes and it is therefore not possible to assess whether an actual e-cigarette exposure situation could result in respiratory sensitisation due to inhalation of cobalt.

Furthermore, a total of 10 substances used in e-liquids has a notified classification with H334, but in general the classification is only notified by a few notifiers. For one substance (thujone), this classification is notified by almost all notifiers (23 of 25). However, no sensitising level was available for this substance and the substance has not been measured in vapour from e-cigarettes, i.e. it was not possible to conclude whether the substance is found in vapours in concentrations leading to respiratory sensitisation.

A total of five substances identified in vapours from e-cigarettes has a notified classification with H334. However, for all five substances, the numbers of notifiers notifying this classification are low and well below 50% of the notifiers.

This elaboration project as well as the two former projects (Poulsen et al., 2017a and 2017b) illustrate that it is necessary to set limit values for frequently used ingredients in e-liquids as well as frequently identified substances in vapours from e-cigarettes, as long-term use of e-cigarettes cannot be considered safe when looking at the maximum identified concentrations in e-liquids and vapours.

11 References

Afnor, 2015b. XP D90-300-2. Electronic cigarettes and e-liquids — Part 2: Requirements and test methods for e-liquids. Afnor, March 2015. https://www.boutique.afnor.org/xml/1855862/false

Afnor, 2016. XP D90-300-3. Electronic cigarettes and e-liquids — Part 3: Requirements and test methods for emissions. Afnor, July 2016. https://www.boutique.afnor.org/xml/1896345/false

AgBB, 2015. AgBB - Evaluation procedure for VOC emissions from building products; February 2015. Health-related Evaluation Procedure for Volatile Organic Compounds Emissions (VVOC, VOC and SVOC) from Building Products. https://www.umweltbundesamt.de/sites/default/files/medien/355/dokumente/agbb_evaluation_scheme_2015.pdf

ANSES, 2007. Valeurs guides de qualité d'air intérieur. Le formaldehyde. Juillet 2007. https://www.anses.fr/en/system/files/AIR2004etVG002Ra.pdf

ANSES, 2010. Activités professionnelles et qualité de l'air des parcs de stationnement couverts. Avis de l'Anses. Rapport d'expertise collective. Juillet 2010. Édition scientifique. https://www.anses.fr/fr/system/files/AIR-Ra-Parking.pdf

ANSES, 2013a. Summary table of published values. Proposed IAQG values. IAQG = Indoor Air Quality Guidelines. Updated March 27, 2013. French Agency for Food, Environmental and Occupational Health & Safety (ANSES). https://www.anses.fr/fr/system/files/ANSES-Ft-VGAIpublieesEN.pdf

ANSES, 2013b. Proposition de valeur guides de qualité d'air intérieur. L'acroléine. Avis de l'Anses. Rapport d'expertise collective. Avril 2013. Edition scientifique. https://www.anses.fr/fr/system/files/AIR2011sa0354Ra.pdf

ANSES, 2014. Proposition de valeurs guides de qualité d'air intérieur. L'acétaldéhyde. Avis de l'Anses. Rapport d'expertise collective. Avril 2014. Edition scientifique. https://www.anses.fr/fr/system/files/AIR2013sa0076Ra.pdf

ANSES, 2017. Élaboration de VTR par voie respiratoire pour le toluene. Avis de l'Anses. Rapport d'expertise collective. Octobre 2017. Édition scientifique. https://www.anses.fr/fr/system/files/SUBSTANCES2017SA0102Ra.pdf

ANSES, 2018. Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on the revision of ANSES's reference values for formaldehyde: occupational exposure limits (OELs), derived no-effect levels (DNELs) for professionals, toxicity reference values (TRVs) and indoor air quality guidelines (IAQGs). 2 February 2018. https://www.anses.fr/en/system/files/SUBSTANCES2017SA0040EN.pdf

Appelman et al., 1982. Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies. Toxicology 23: 293–307, 1982. Appelman LM, Woutersen RA and Feron VJ.

Appelman et al., 1986. Effect of variable versus fixed exposure levels on the toxicity of acetaldehyde in rats. J. Appl. Toxicol. 6: 331–336, 1986. Appelman LM, Woutersen RA, Feron VJ, Hoofman RN and Notten WRF.

ATSDR, 1997. Toxicological profile for Propylene Glycol CAS 57-55-6, ATSDR (Agency for Toxic Substances and Disease Registry). September 1997. https://www.atsdr.cdc.gov/ToxProfiles/tp189.pdf

ATSDR, 1999. Toxicological profile for formaldehyde. US. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR). July 1999. https://www.atsdr.cdc.gov/toxprofiles/tp111.pdf

ATSDR, 2004. Toxicological profile for cobalt. US Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. April 2004. https://www.atsdr.cdc.gov/toxprofiles/tp33.pdf

ATSDR, 2007a. Toxicological profile for Acrolein. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR). August 2007. https://www.atsdr.cdc.gov/ToxProfiles/tp124.pdf

ATSDR, 2007b. Toxicological profile for xylene. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR). August 2007. https://www.atsdr.cdc.gov/toxprofiles/tp71.pdf

ATSDR, 2010. Toxicological profile for Ethylene Glycol CAS 107-21-1, US. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR). November 2010. https://www.atsdr.cdc.gov/toxprofiles/tp96.pdf

ATSDR, 2017. Toxicological profile for toluene. U.S. Department of Health and Human Services, Public Health Service. Agency for Toxic Substances and Disease Registry, June 2017. https://www.atsdr.cdc.gov/toxprofiles/tp56.pdf

BfR, 2012. Liquids from e-cigarettes can be detrimental to health. BfR Opnion no. 016/2012, 24 February 2012. http://www.bfr.bund.de/cm/349/liquids-from-e-cigarettes-can-be-detrimental-to-health.pdf

BSI, 2015. PAS 54115:2015. Vaping products, including electronic cigarettes, e-liquids, e-shisha and directly-related products – Manufacture, importation, testing and labelling – Guide. British Standards Institution. July 2015.

Cheng, 2014. Chemical evaluation of electronic cigarettes. Cheng T. Tob control 2014; 23: ii11-ii17. http://tobaccocontrol.bmj.com/content/23/suppl_2/ii11.full

CIR, 2015. Safety Assessment of Glycerin as Used in Cosmetics. Final Report, January 14, 2015. Cosmetic Ingredient Review.

Colard et al., 2015. Electronic Cigarettes and Indoor Air Quality: A Simple Approach to Modeling Potential Bystander Exposures to Nicotine. Colard S, O'Conell G, Verron T, Cahours X, Pritchard JD. Int. J. Environ. Res. Public Health 2015, 12, 282-299. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4306862/

Coon et al., 1970. Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. Toxicol Appl Pharmacol 16, 646-655, 1970. Coon RA, Jones RA, Jenkins LJ and Siegel J.

DHI, 2016. Cosmetic Product Safety Report on Dhiva Shampoo for Dhiva Cosmetics. October 2016.

Dorman et al., 2008a. Derivation of an inhalation reference concentration based upon olfactory neuronal loss in male rats following subchronic acetaldehyde inhalation. Inhal. Toxicol. 20, 245-256, 2008. Dorman DC, Struve MF, Wong BA, Gross EA, Parkinson C, Willson GA, Tan YM, Campbell JL, Teeguarden JG, Clewell HJ, III, Andersen ME.

Dorman et al., 2008b. Respiratory tract responses in male rats following subchronic acrolein inhalation. Inhal Toxicol. 2008 Feb;20(3):205-16. Dorman DC, Struve MF, Wong BA, Marshall MW, Gross EA, Willson GA.

Drummond et al., 2014. Electronic Cigarettes. Potential Harm and Benefits. Drummond MB and Upson D. AnnaIsATS Volume 11, Number 2, February 2014. https://www.ncbi.nlm.nih.gov/pubmed/24575993

ECHA, 2012. Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health. https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf

ECHA, 2018. Searches in the ECHA database “Information on chemicals”, mainly data for registered substances. https://echa.europa.eu/information-on-chemicals

EMA, 2012. Public statement on the use of herbal medicinal products containing thujone. Final. 22 May 2012. EMA/HMPC/732886/2010 Rev.1. Committee on Herbal Medicinal Products (HMPC), European Medicines Agency. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/02/WC500123136.pdf

EMA, 2017. Propylene glycol used as an excipient. Report published in support of the ‘Questions and answers on propylene glycol used as an excipient in medicinal products for human use’ (EMA/CHMP/704195/2013). 9 October 2017. EMA/CHMP/334655/2013. Committee for Human Medicinal Products (CHMP). European Medicines Agency. http://www.ema.europa.eu/docs/en_GB/document_library/Report/2017/10/WC500235913.pdf

Environment Canada, 2000a. Priority Substances List Assessment Report. Canadian Environmental Protection Act, 1999. Acetaldehyde. Environment Canada, Health Canada, May 2000. http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/psl2-lsp2/acetaldehyde/acetaldehyde_fin-eng.pdf

Environment Canada, 2000b. Priority Substances List Assessment Report. Canadian Environmental Protection Act, 1999. Acrolein. Environment Canada, Health Canada, May 2000. http://publications.gc.ca/Collection/En40-215-48E.pdf

Environment Canada, 2007. Priority Substances List Assessment Report. Follow-up to the State of Science Report, 2000. Ethylene Glycol. Environment Canada. Health Canada. December 2007. https://www.ec.gc.ca/lcpe-cepa/documents/substances/eg/eg_draft-eng.pdf

EU Directive no. 95, 2001. Directive 2001/95/EC of the European Parliament and of the Council of 3 December 2001 on general product safety. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02001L0095-20100101&from=EN

EU Directive no. 40, 2014. Directive 2014/40/EU of the European Parliament and of the Council of 3 April 2014 on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco and related products and repealing Directive 2001/37/EC. Consolidated version dated 2015.01.06. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02014L0040-20150106&qid=1484576281674&from=EN

EU-LCI, 2016. Agreed EU-LCI values. December 2016. European Commission. http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=4&cad=rja&uact=8&ved=2ahUKEwjAqeaJ8vPcAhUMMuwKHUm0B4sQFjADegQIBxAC&url=http%3A%2F%2Fec.europa.eu%2FDocsRoom%2Fdocuments%2F22321%2Fattachments%2F1%2Ftranslations%2Fen%2Frenditions%2Fnative&usg=AOvVaw10SxapgUxkf3i0c9O9MCyB

EU-LCI, 2018. Agreed EU-LCI values. July 2018. European Commission. http://ec.europa.eu/growth/sectors/construction/eu-lci/values_en

Farsalinos et al., 2015a. Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins. Farsalinos KE, Kistler KA, Gillman G, Voudris V. Nicotine Tob Res. 2015 Feb; 17(2):168-74. https://academic.oup.com/ntr/article-abstract/17/2/168/2858003/Evaluation-of-Electronic-Cigarette-Liquids-and

Farsalinos et al., 2015b. Are Metals Emitted from Electronic Cigarettes a Reason for Health Concern? A Risk-Assessment Analysis of Currently Available Literature. Farsalinos KE, Voudris V, Poulas K. Int. J. Environ. Res. Public Health, 2015, 12(5), p. 5215-5232. http://www.mdpi.com/1660-4601/12/5/5215

Fiume et al., 2012. Safety Assessment of Propylene Glycol, Tripropylene Glycol, and PPGs as Used in Cosmetics. Int. Journal of Toxicology 31 (Supplement 2), 245S-260S, 2012. Fiume MM, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler D, Marks JG, Shank RC, Slaga TJ, Snyder PW, Andersen FA. https://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/PR560.PDF

Foo et al., 1990. Chronic neurobehavioural effects of toluene. British journal of industrial medicine, 47: 480–484. Foo SC, Jeyaratnam J, Koh D. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1035211/pdf/brjindmed00043-0048.pdf

Foo et al., 1993. Neurobehavioural effects in occupational chemical exposure. Environmental research, 60: 267–273, 1993. Foo SC, Ngim CH, Salleh I, Jeyaratnam J, Boey KW. https://www.sciencedirect.com/science/article/pii/S0013935183710364

Goniewicz et al., 2014. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, Prokopowicz A, Jablonska-Czapla M, Rosik-Dulewska C, Havel C, Jacob P III, Benowitz N. Tob Control 2014;23:133–139.

Hahn et al., 2014. Electronic cigarettes: overview of chemical composition and exposure estimation. Hahn J, Monakhova YB, Hengen J, Kohl-Himmelseher M, Schüssler J, Hahn H, Kuballa T, Lachenmeier DW. Tobacco Induced Diseases (2014) 12:23. https://tobaccoinduceddiseases.biomedcentral.com/articles/10.1186/s12971-014-0023-6

Hutzler et al., 2014. Chemical hazards present in liquids and vapors of electronic cigarettes. Hutzler C, Paschke M, Kruschinski S, Henkler F, Hahn J, Luch A. Arch Toxicol (2014) 88:1295–1308.

Korsak et al., 1994. Toxic effects of subchronic combined exposure to n-butyl alcohol and m-xylene in rats. Int J Occup Med Environ Health 7:155-166. Korsak Z, Wisniewska-Knypl J, Swiercz, R, 1994.

Lang et al., 2008. Formaldehyde and chemosensory irritation in humans: a controlled human exposure study. Regul Toxicol Pharmacol. 2008 Feb; 50(1): 23-36. Epub 2007 Sep 5. Lang I, Bruckner T, Triebig G. https://www.ncbi.nlm.nih.gov/pubmed/17942205

Michigan DEQ, 2015. Michigan Department of Environmental Quality. File for Benzophenone (CAS No. 119-61-9). Michael Depa, Toxics Unit, Air Quality Division. July 10, 2015 http://www.deq.state.mi.us/aps/downloads/ATSL/119-61-9/119-61-9_annual_ITSL_IRSL.pdf

Mikkelsen et al., 2015. Survey and health assessment of UV filters. Survey of chemical substances in consumer products. No. 142, 2015. Danish EPA. Mikkelsen SH, Lassen C, Warming M, Hansen E, Brinch A, COWI. Brooke D, Crookes M, Building Research Establishment Ltd. Nielsen E, Bredsdorff L, DTU Food. https://www2.mst.dk/Udgiv/publications/2015/10/978-87-93352-82-7.pdf

OEHHA, 2016. The Office of Environmental Health Hazard Assessment (OEHHA), California, USA. https://oehha.ca.gov/air/general-info/oehha-acute-8-hour-and-chronic-reference-exposure-level-rel-summary

NICNAS, 2013. Human Health Tier II Assessment for xylenes Australian Government. Department of Health. National Industrial Chemical Notification and Assessment Scheme (NICNAS). Year of publication of website unknown, but accessed in 2017 and newest used reference from 2013. https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=126

NICNAS, 2014. Human Health Tier II Assessment for ethanedial, CAS Number: 107-22-2. Australian Government. Department of Health. National Industrial Chemical Notification and Assessment Scheme (NICNAS). Year of publication of website unknown, but accessed in 2017 and newest used reference from 2014. https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-assessment-details?assessment_id=73 https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-assessment-details?assessment_id=73#health

NIEHS, 2002. Cobalt Dust [7440-48-4]. Review of Toxicological Literature. February 2002. Prepared for National Institute of Environmental Health Sciences. https://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/cobaltdust_508.pdf

Nielsen & Ladefoged, 2013. Evaluation of health hazards by exposure to Ethylene glycol and proposal of a health-based quality criterion for ambient air. Environmental Project No. 1495, 2013, Danish EPA. Nielsen E, Ladefoged O, Division of Toxicology and Risk Assessment. National Food Institute, Technical University of Denmark. https://www2.mst.dk/Udgiv/publications/2013/08/978-87-93026-32-2.pdf

NIOSH, 2016. Criteria for a recommended standard: occupational exposure to diacetyl and 2,3-pentanedione. By McKernan LT, Niemeier RT, Kreiss K, Hubbs A, Park R, Dankovic D, Dunn KH, Parker J, Fedan K, Streicher R, Fedan J, Garcia A, Whittaker C, Gilbert S, Nourian F, Galloway E, Smith R, Lentz TJ, Hirst D, Topmiller J, Curwin B. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2016-111.

NTP, 2017. Toxicity effects. CAS No. 76231-76-0. National Toxicology Program. US Department of Health and Human Services. https://tools.niehs.nih.gov/cebs3/ntpviews/index.cfm?action=testarticle.toxicity&cas_number=76231-76-0

OECD SIDS, 2001. 1,2-Dihydroxypropane. CAS: 57-55-6. http://www.inchem.org/documents/sids/sids/57-55-6.pdf

OECD SIDS, 2002a. Glycerol, CAS no. 56-81-5. SIDS Initial Assessment Report For SIAM 14, Paris, France, 26-28 March 2002, UNEP Publications. http://www.inchem.org/documents/sids/sids/56815.pdf

OECD SIDS, 2002b. Linalool. CAS No. 78-70-6. SIDS Initial Assessment Report For SIAM 14, Paris, France, 26-28 March 2002, UNEP Publications. http://www.inchem.org/documents/sids/sids/78706.pdf

OECD SIDS, 2003. SIDS Initial Assessment Report (SIAR) on glyoxal, CAS No. 107-22-2. http://www.inchem.org/documents/sids/sids/107222.pdf

OECD SIDS, 2004. SIDS Initial Assessment Profile on Ethylene Glycols (Ethylene glycol, Diethylene glycol, Triethylene glycol, Tetraethylene glycol, Pentaethylene glycol). SIAM 18, 20-23 April 2004. https://hpvchemicals.oecd.org/UI/handler.axd?id=0551d8c1-6c0e-4886-be62-b3d3fa8fc49a

OECD SIDS, 2007. SIDS Dossier on the HPV Chemical Ethylene Glycol, CAS No. 107-21-1. https://hpvchemicals.oecd.org/ui/SIDS_Details.aspx?id=aacf6f16-58aa-4801-ac76-4437e9b62ed4

Pisinger et al., 2014. A systematic review of health effects of electronic cigarettes. Pissinger C, Døssing M. Pisinger et al., 2014, 248-260. https://www.ncbi.nlm.nih.gov/pubmed/25456810

Poulsen et al., 2017a. Requirements for substances in e-liquids used in electronic cigarettes. FORCE Technology, Poulsen PB, Merlin C, Schmidt A. Commissioned by The Consumer Council at the Austrian Standards Institute. November 2017.

Poulsen et al., 2017b. Requirements for substances formed or released during the evaporation of e-liquids used in electronic cigarettes. FORCE Technology, Poulsen PB, Merlin C. and Schmidt A. November 2017. Commissioned by The Consumer Council at the Austrian Standards Institute.

SCCP, 2005. SCCP (Scientific Committee on Consumer Products). 2005. Opinion on Glyoxal. SCCP/0881/05.
http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_023.pdf

SCF, 2003. Opinion of the Scientific Committee on Food on Thujone (expressed on 2 December 2002). SCF/CS/FLAV/FLAVOUR/23 ADD2 Final. 6 February 2003. European Commission. Health & Consumer Protection Directorate-General. Scientific Committee on Food.
https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out162_en.pdf

Suber RL, Deskin R, Nikiforov I, Fouillet X, Coggins CR, 1989. Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. Food Chem Toxicol. 27(9):573-83.

Tayyarah & Long, 2014. Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air. Regulatory Toxicology and Pharmacology 70 (2014) 704–710.
http://www.sciencedirect.com/science/article/pii/S0273230014002505

TOXNET, 2018. Searches performed on https://toxnet.nlm.nih.gov/ in august 2018.

UBA, 2016. Website on German Committee on Indoor Guide Values.
https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/german-committee-on-indoor-guide-values
Guide Values (I and II) – table:
https://www.umweltbundesamt.de/sites/default/files/medien/355/bilder/dateien/0_ausschuss_fur-innenraumrichtwerte_empfehlungen_und_richtwerte20170210_en.pdf

UBA, 2018. Website on German Committee on Indoor Guide Values.
https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/german-committee-on-indoor-guide-values#textpart-1
Guide Values (I and II) – table:
https://www.umweltbundesamt.de/sites/default/files/medien/355/bilder/dateien/0_ausschuss_fur-innenraumrichtwerte_empfehlungen_und_richtwerte_20180412_en.pdf

Uchida et al., 1993. Symptoms and signs in workers exposed predominantly to xylenes. Int. Arch. Occup. Environ. Health 64:597-605. Uchida Y, Nakatsuka H, Ukai H, Watanabe T, Liu YT, Huang MY et al. 1993.

Uchiyama et al., 2014. Determination of Carbonyl Compounds Generated from the E-cigarette Using Coupled Silica Cartridges Impregnated with Hydroquinone and 2,4-Dinitrophenylhydrazine, Followed by High-Performance Liquid Chromatography. Uchiyama S, Ohta K, Inaba Y, Kunugita N. Analytical Sciences December 2013, Vol. 29.

US EPA, 1987. Ethylene glycol; CASRN 107-21-1. Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency. Chemical Assessment Summary National Center for Environmental Assessment. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0238_summary.pdf

US EPA, 2008. Provisional Peer Reviewed Toxicity Values for Propylene glycol (CASRN 57-55-6). Superfund Health Risk Technical Support Center, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268. https://cfpub.epa.gov/ncea/pprtv/documents/PropyleneGlycol.pdf

Varlet et al., 2015. Toxicity Assessment of Refill Liquids for Electronic Cigarettes. Varlet V, Farsalinos K, Augsburger M, Thomas A, Etter J-F. Int. J. Environ. Res. Public Health 2015, 12, 4796-4815. http://www.mdpi.com/1660-4601/12/5/4796

Visser et al., 2015. The health risks of using e-cigarettes. Visser W, Geraets L, Klerx W, Hernandez L, Stephens E, Croes E, Schwillens P, Cremers H, Bos P, Talhout R. RIVM Report 2015-0144. National Institute for Public Health and the Environment. http://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2015/juli/The_health_risks_of_using_e_cigarettes

WHO, 2000. Air Quality Guidelines for Europe, Second Edition. WHO Regional Publications, European Series, No. 91. WHO, Regional Office for Europe, Copenhagen.

WHO, 2002. Concise International Chemical Assessment Document 45. Ethylene Glycol: Human Health Aspects. World Health Organization, Geneva, 2002. http://www.who.int/ipcs/publications/cicad/en/cicad45.pdf

WHO, 2004. Concise International Chemical Assessment Document (CICAD) 57: Glyoxal. Human Health Aspects. World Health Organization, Geneva, 2004. http://www.inchem.org/documents/cicads/cicads/cicad57.htm#6.2.1

WHO, 2010. WHO Guidelines for Indoor Air Quality. Selected Pollutants. WHO, Europe. http://www.euro.who.int/__data/assets/pdf_file/0009/128169/e94535.pdf

Williams et al., 2013. Metal and Silicate Particles Including Nanoparticles Are Present in Electronic Cigarette Cartomizer Fluid and Aerosol. Williams M, Villarreal A, Bozhilov K, Lin S, Talbot P. PLoS ONE 8(3): e57987. http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0057987&type=printable

Wills et al., 1974. Inhalation of aerosolised ethylene glycol by man. Clin Toxicol 7: 463-476. Wills JH, Coulston F, Harris ES, McChesney EW, Russel JC, Serrone DM. https://www.tandfonline.com/doi/abs/10.3109/15563657408988020

Zavalic et al., 1998. Quantitative assessment of color vision impairment in workers exposed to toluene. American journal of industrial medicine. 1998 Mar;33(3):297-304. Zavalić M, Mandić Z, Turk R, Bogadi-Sare A, Plavec D.