Requirements for substances in e-liquids used in electronic cigarettes

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Summary

Smoking by use of electronic cigarettes (e-cigarettes) is a new phenomenon. BfR, the German Federal Institute for Risk Assessment, prepared in 2012 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 in the e-liquids such as solvents (propylene glycol, glycerine), various scent and aroma substances (e.g. menthol, linalool) and contaminants etc. can pose health risks as well.

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 chemical substances in e-liquids are limited except for a requirement of the level of nicotine in the e-liquids. Standards are now being developed in relation to CEN/TC 437 “Electronic cigarettes and e-liquids”, which was established in 2015.

The purpose of this study was to develop a proposal for requirements for selected substances in e-liquids based on relevant existing threshold values for different chemical substances. The focus will be on the substances in e-liquids and not the substances formed by the vaporisation. Furthermore, the purpose of the study is to review existing standards on e-cigarettes, focusing on requirements for e-liquids.

This project is carried out as a desk-top study and includes a description of existing legislation regarding chemical substances in e-liquids and a review of the chemical requirements in existing standards on e-liquids. Furthermore, a screening (an internet search) for ingredients used in e-liquids has been prepared and relevant existing threshold limit values (TLVs) for these ingredients were identified. Finally, 15 ingredients were selected for which a proposal for limit values of these ingredients in e-liquids has been calculated based on the identified TLVs and based on simple ‘worst case’ calculations. The proposed preliminary calculated limit values for the 15 selected ingredients in e-liquids are presented in chapter 8 “Preliminary proposal for limit values of selected ingredients”.

This project only covers the ingredients used in e-liquids. Substances formed during the evaporation of e-liquids are dealt with in another project.

It must be emphasised that the calculations performed in this report are made on a screening level, i.e. that a simple ‘worst case’ calculation has been made. This means that it is assumed 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. Absorption of the substances in the body may therefore be overestimated and the proposed limit values may therefore be lower than necessary. However, in case of missing information regarding absorption a precautionary principle should be used. Furthermore, it can also be discussed which limit values that should be used for the individual substances. In this report, the lowest limit values found have generally been used. The proposed preliminary limit values in this report should therefore be considered as a first approximation and the risk assessment presented needs further refinement in order to establish limit values for ingredients in e-liquids.

The calculated proposed limit values for the 15 selected ingredients in e-liquids lie between 0.012 µg/mL e-liquid (for nickel) and 78,000 µg/mL e-liquid corresponding to 7.8 % (for menthol).

For 10 of the 15 substances where limit values in e-liquids are proposed, information about the actual measured concentration in e-liquids has been identified in this review. In 9 of 10 cases, the actual identified maximum concentration in e-liquids is higher than the proposed limit value. In some cases, the maximum concentrations found in e-liquids are a factor of 2-4 times higher than the proposed limit values in this report. In other cases, the maximum concentration found in e-liquids exceeds the proposed limit value by a factor of 25 to 200 – in one case even by a factor of 19,000 (for nickel). This fact suggests that limit values may be needed.

However, as the available information about the actual minimum concentrations measured in e-liquids reveals, it will be possible for some of the examined e-liquids to meet the proposed limit values for 8 of the 10 substances where measurements have been carried out. For example, benzaldehyde was only identified in 4 out of 28 e-liquids where its content was measured. This suggests that it will be possible for a large part of e-liquids on the market to meet the suggested limit value for benzaldehyde. Furthermore, the substances diacetyl and acetyl propionyl have been identified in e-liquids in concentrations below the proposed limit values. The extent of e-liquids below the proposed limit values is, however, unknown.

Some of the most frequently used ingredients and the ingredients used in the highest concentration (sometimes up to 79%) – are the solvents glycerine, propylene glycol and ethylene glycol. The maximum measured concentrations of these solvents also exceed the proposed limit values (based on 500 puffs/day) by a factor of 26, 79 and 48 respectively for the listed solvents. Only for ethylene glycol, the minimum identified measured concentrations in e-liquids of 1% is below the proposed limit value of 1.6%. However, according to the Afnor standard (2015b), ethylene glycol is restricted with ‘no use’. This suggests that the use of the solvents in e-liquids needs further examination.

In contrast, the elements (lead and cadmium) are exceed by a factor of 2 to 3.4, i.e. the maximum identified concentrations (impurities) in e-liquids are just exceeding the proposed limit values. It must, however, be emphasised that for impurities such as lead, cadmium and nickel, it was only in about 3 to 15% of the measured cases that a content of these elements was identified above the level of quantification (LOQ), 5, 1 and 10 ng/mL respectively. Furthermore, it must be stressed that the proposed limit values for these three elements are all above the used level of detection. This indicates that most of the e-liquids could comply with the proposed limit values for the elements lead, cadmium and nickel.

According to the Tobacco Directive, it is only allowed to use ingredients in e-cigarettes and e-liquids, which do not pose a risk to human health in heated and unheated form. This review and other reviews on the subject illustrate that ingredients are used in concentrations, which cannot be considered as safe. This fact emphasises the need for limit values for several of the ingredients used in e-liquids.

Neither the GPS Directive nor the Tobacco Directive contain specific requirements for e-cigarettes and e-liquids. It is therefore important that the standards developed set the necessary chemical requirements (for all the necessary chemical substances

contained in the e-liquids) as well as the correct limit values to protect the consumer from unwanted health effects from ingredients contained in e-liquids.

1 Introduction

1.1 Background

Smoking by use of electronic cigarettes (e-cigarettes) is a new phenomenon. BfR, the German Federal Institute for Risk Assessment, prepared in 2012 an opinion on liquids in e-cigarettes (BfR, 2012) 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 as well. 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.

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 chemical substances in 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.

1.2 Purpose

The purpose of this study was to develop a proposal for requirements for selected substances in e-liquids based on relevant existing threshold values for different chemical substances. The focus will be on the substances in e-liquids and not the substances formed by the vaporisation. Furthermore, the purpose of the study is to review existing standards on e-cigarettes, focusing on requirements for e-liquids.

1.3 Definitions

According to the Tobacco Directive (EU Directive no. 40, 2014), ‘electronic cigarettes’ or ‘**e-cigarettes**’, which is the term used in this report, is defined as: “means 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 **ingredients**:

1. A diluent (propylene glycol and/or glycerol and perhaps water)
2. Nicotine, CAS 54-11-5 (may be added)
3. A flavouring compound (may be added)
4. Other ingredients (may be added)

The diluent currently consists mainly of propylene glycol (CAS 57-55-6) and glycerol (CAS 56-81-5) (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. According to Hutzler et al., 2014, which carried out a chemical analysis of 28 different e-cigarettes, 10 out of 28 e-cigarettes were declared as being “free-of-nicotine”, which means that 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 (CAS 121-33-5), menthol (CAS 89-78-1) and trimethylpyrazine (CAS 14667-55-1) according to the findings in this report.

Other ingredients can be either deliberately added ingredients such as the pharma ingredient rimonabant (CAS 158681-13-1) or it can be impurities such as tabacco-specific nitrosamines (TSNA) and different metals.

2 Project methodology used

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

1. Description of existing legislation regarding chemical substances in e-liquids
2. Review of existing standards on e-liquids in electronic cigarettes – with a focus on the chemical requirements concerning the ingredients
3. Screening of ingredients (chemical substances) in e-liquids
4. Identification of relevant existing threshold values for ingredients
5. Prioritisation and selection of ingredients for which a proposal for limit values will be calculated
6. Proposal for requirements for selected substances in e-liquids
7. Discussion, conclusion and recommendation

The methodology used for the different tasks is described briefly below.

Description of existing legislation regarding chemical substances in e-liquids
The legislation relevant for chemical substances in e-liquids is described and reviewed in chapter 3. The legislation reviewed is the CLP Regulation, the General Product Safety Directive and the Tobacco Directive.

Review of existing standards on e-liquids in electronic cigarettes
Currently, standards are under development in relation to CEN/TC 437 "Electronic cigarettes and e-liquids", which was established in 2015. However, no standards are available so far. For this reason, national standards on e-cigarettes have been reviewed in chapter 4.

Screening of ingredients (chemical substances) in e-liquids
A screening of chemical substances (ingredients) used in e-liquids was performed by searching for articles on the subject on the internet. Furthermore, the project advisor contributed with a list of articles found on the internet on the subject. Only articles addressing the ingredients used in e-liquids were used. A list of ingredients used in e-liquids is presented in chapter 5.

Proposal for requirements for selected substances in e-liquids
In chapter 5 relevant existing threshold values for the listed identified ingredients have been identified as well. This information has been used to prioritise (chapter 6), which ingredients that should be selected for the main task of this report, i.e. to propose requirements for the selected substances in e-liquids (chapter 8).

Discussion, conclusion and recommendation
The results of the report are discussed and conclusions made in chapter 9.

3 Legislation

The following relevant legislation regarding the use of chemical substances in e-liquids in e-cigarettes will be described:

- CLP Regulation
- General Product Safety Directive (GPSD)
- Tobacco Directive

This legislation will be described and discussed below, but only with the focus on requirements for chemical substances used in e-liquids for e-cigarettes.

3.1 CLP Regulation

The CLP Regulation (EU Regulation no. 1272, 2008) on classification, labelling and packaging of substances and mixtures does not set chemical requirements for substances in e-liquids, but describes the legal requirements concerning classification and labelling of chemical mixtures that contain dangerous chemical substances. This means that the CLP Regulation describes how the e-liquids should be classified and labelled depending on the content of dangerous chemical substances. Regarding the labelling requirements, only the chemical substances which contribute or lead to a classification need to be indicated on the label. However, certain substances (e.g. sensitizers) need to be labelled even though the mixture is not classified as dangerous.

In the French standard for e-cigarettes (Afnor, 2015b), it is required that an e-liquid is formulated in such a way that, without nicotine, it shall not be classified as hazardous according the CLP Regulation.

If an e-liquid contains nicotine, a report from Bibra (Bibra, 2014) prepared for ECITA (Electronic Cigarette Industry Trade Association) has concluded that a nicotine concentration below 2.5% will not result in a classification as dangerous. As the maximum concentration allowed in e-liquid is 20 mg/ml corresponding to 2%, the e-liquids will not be classified today because of the nicotine content. This conclusion is, however, based on the existing classification of nicotine of Acute Tox. 3*, H301 “Toxic if swallowed”, Acute Tox. 1, H310 “Fatal in contact with skin” and Aquatic Chronic 2, H411 “Toxic to aquatic life with long lasting effects”. The ‘*’ indicates that this is a minimum classification and requires manufacturers and importers of nicotine to investigate whether he has access to data or other information that leads to a more severe category and applies this more severe category.

A CLH report prepared for ECHA for nicotine (RIVM, 2015) has suggested that the Acute Tox. 3*, H301 classification should be replaced by Acute Tox. 1, H300 “Fatal if swallowed“, which is based on lower LD50 values for other test animals. A more severe classification will of course alter the concentration for which a nicotine content will result in a classification as dangerous for the entire e-liquid mixture. A classification with Acute Tox. 1, H300 for nicotine will result in a concentration of 0.1% for nicotine being the limit value for classification as dangerous, which would imply that nicotine-containing e-liquids in the future may be classified as dangerous.

3.2 Tobacco Directive

The Tobacco Directive (EU Directive no. 40, 2014) lays down the rules for ingredients and emissions of tobacco products including e-cigarettes, as well as rules for labelling. Both e-cigarettes and refill containers (e-liquids) are covered by the Tobacco Directive.
The legislation concerning e-cigarettes and e-liquids in the Tobacco Directive entered into force by November 20, 2016.

The legislation concerning e-cigarettes is described in Article 20 "Electronic cigarettes" and contains the following elements:

- Notification to competent authorities of the Member States
- Requirements concerning nicotine
- General requirements concerning ingredients
- Requirements concerning child- and tamper-proof refill containers
- Labelling, instructions for use and health warnings
- Requirements concerning submission of sales volumes and other information to the competent authorities of the Member States
- General requirements concerning the Member States and gathering of information on the use of e-cigarettes

Only the rules concerning chemical requirements to ingredients in e-liquids will be described in detail below.

All manufactures and importers of e-cigarettes and refill containers (e-liquids) must submit a **notification** to the competent authorities of the Member States with the following information:

- Name and contact details of the manufacturer/importer.
- A list of all ingredients contained in and emissions resulting from the use of the product, including quantities.
- Toxicological data regarding the ingredients and emissions including when heated. In particular, the health effects of the ingredients when inhaled by consumers and taking into account any addictive effect.
- Information on nicotine doses and absorption, when consumed under normal or reasonably foreseeable conditions.
- A description of the components of the product, including a description of the opening and refill mechanism.
- A description of the production process.
- A declaration stating that the manufacturer and importer bear full responsibility for the quality and safety of the product, when placed on the market and used under normal or reasonably foreseeable conditions.

Requirements concerning nicotine are:

- Nicotine-containing liquids must not contain more than 20 mg/ml nicotine.
- The e-cigarettes must deliver the nicotine doses at consistent levels under normal conditions of use.

Besides requirements for nicotine, some **general requirements concerning the ingredients** are listed:

- Only ingredients of high purity must be used in the manufacturing of the nicotine-containing e-liquids.
- Only trace levels of other ingredients than listed for the e-liquid are allowed and only if technically unavoidable during manufacture.

- Except for nicotine, only ingredients which do not pose a risk to human health in heated or unheated form must be used.
- Nicotine-containing liquids must not contain the following additives:
    - Vitamins or other additives that create the impression that the product has a health benefit
    - Caffeine or taurine or other additives and stimulant compounds that are associated with energy and vitality
    - Additives having colouring properties for emissions
    - Additives that have CMR[1] properties in unburnt form

According to the **labelling instructions**, the outside packaging of e-cigarettes and refill containers must contain a list of all ingredients contained in the product in descending order of weight. Furthermore, it must be indicated, what the nicotine content is and the delivery per dose. Health warnings concerning nicotine must be listed on the outside packaging of e-cigarettes and e-liquids.

Finally, **the Member States must ensure**:

- That these requirements also apply to cross-border distance sales of e-cigarettes and e-liquids.
- That a system for collecting information about all suspected adverse effects on human health of these products are established and maintained.
- Products which are not safe or could present a serious risk to human health or not of good quality are withdrawn (and perhaps recalled) from the market.

3.3 General Product Safety Directive (GPSD)

The General Product Safety Directive (EU Directive no. 95, 2001) is intended to ensure product safety for EU consumer products not covered by specific other legislation, by stating that "producers shall be obliged to place safe products on the market".

Safe products are defined as "any product, which under normal or reasonably foreseeable conditions of use… does not present any risk… considered to be acceptable with a high level of protection for the safety and health of persons…". Products are presumed safe:

1. If they follow specific Community provisions concerning safety of the product. This means that the General Product Safety Directive does only apply to risks or categories of risks not covered by the specific European product legislation if specific European product legislation, such as the Tobacco Directive, applies. However, article 5 to 18 of the GPSD (concerning e.g. market surveillance from Member States, RAPEX notifications as well as producers' and distributors' obligation to inform consumers of risks, i.e. by use of appropriate warnings) still apply even though the Tobacco Directive also applies.
2. If they follow specific national legislation concerning safety of the product.
3. If they follow European standards, the references which have been published by the Commission in the Official Journal of the European Communities.
4. If they follow other European Standards.
5. If they follow relevant national standards (which do not transpose European standards).

[1] CMR = Carcinogenic, Mutagenic or toxic to Reproduction

6. If, in the absence of product specific legislation or relevant national standards, they follow:
    - Commission recommendations setting guidelines on product safety,
    - product safety codes of good practice in force for the sector concerned,
    - the state of art and technology
    - and reasonable consumer expectations concerning safety.

Furthermore, producers must inform consumers of the risks associated with the products they supply.

The Tobacco Directive only applies for nicotine containing e-cigarettes, whereas e-cigarettes not containing nicotine are not covered. For such products, only the GPS Directive applies. The safety assessment of the ingredients in accordance with the GPS Directive may rely on requirements set in existing standards (which are under development (in 2017)).

3.4 Discussion on legislation regarding chemical substances in e-liquids

The Tobacco Directive contains specific requirements for e-cigarettes and e-liquids, but 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,
- and a general requirement of no use of ingredients that pose a risk to human health (except for nicotine) or are classified as CMR.

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, which only uses a general statement saying that products on the market ‘must be safe’ (including chemical safety).

Even though the Tobacco Directive contains chemical requirements, these requirements are on a very general level and are rather vague, as it is debatable how to interpret phrases such as “consistent levels”, “high purity”, “trace levels” and “do not pose a risk to human health”.

In practice, this means that it is difficult for the producers of e-liquids (and e-cigarettes) to ensure ‘safe’ e-liquid products on the market, as the general vague requirement of ‘must not pose a risk to human health’ requires risk assessment expertise. From a producer’s point of view, it would be easier to follow limit values of ingredients in e-liquids.

Currently, standards are under development in relation to CEN/TC 437 “Electronic cigarettes and e-liquids”, which was established in 2015. It is important that these standards address the health aspects (chemical safety) of the ingredients used in e-liquids as the products may be considered safe when European Standards are followed.

4 Standards

Currently, standards are under development in relation to CEN/TC 437 "Electronic cigarettes and e-liquids", which was established in 2015. So far (June 2017), no standards have been published on the website of CEN[2] under this committee – neither drafts nor standards under approval. However, some national standards on e-cigarettes and e-liquids do exist, which are also used in the CEN/TC 437 committee as a basis of their standardization work:

- The British document PAS 54115:2015 "Vaping products, including electronic cigarettes, e-liquids, e-shisha and directly-related products – Manufacture, importation, testing and labelling – Guide" (BSI, 2015).
- The French standard XP D 90-300:
    - XP D 90-300-1: "Electronic cigarettes and e-liquids – Part 1: Requirements and test methods for electronic cigarettes" (Afnor, 2015a).
    - XP D 90-300-2: "Electronic cigarettes and e-liquids – Part 2: Requirements and test methods for e-liquids" (Afnor, 2015b).
    - XP D 90-300-3: "Electronic cigarettes and e-liquids – Part 3: Requirements and test methods for emissions" (Afnor, 2016).

These standards will be described and reviewed in this chapter.

4.1 British document PAS 54115

It is written in PAS 54115 that the document is not to be regarded as a British Standard. The PAS document is a guidance document and contains recommendations, and it is emphasised that claims of compliance cannot be made to the document as well as compliance with the PAS document does not imply immunity from legal requirements (BSI, 2015).

The PAS document covers the following areas:

- Purity of e-liquid ingredients in manufacture
- Contaminants arising from device materials and potential emissions from device operations
- Electrical safety
- Metals and carbonyls in emissions

Only the recommendations/requirements that are relevant for the chemical substances contained in e-liquids are described below, i.e. the recommendations for good manufacture practice is not described.

Concerning the **quality of the ingredients**, the PAS document describes the following recommendations:

- The diluent solvents making up the base liquid (that dilutes the concentration of nicotine and/or flavourings) should be of a pure technical grade (European Pharmacopoeia/United States Pharmacopeia Standard (Ph. Eur./USP) grade).

[2] https://standards.cen.eu/dyn/www/f?p=204:105:0

- Propylene glycol should be tested to ensure that the level of di-ethylene glycol (potential contaminant) does not exceed 0.1%.
- Flavouring ingredients should be of Ph. Eur./USP grade or of food grade.
- Regularly monitoring should be carried out in order to ensure that any contaminants are minimised.

The PAS document contains the following recommendations concerning **product toxicological risk assessment** (TRA):

- If justified, a toxicological risk assessment (TRA) should be carried out on ingredients used in the manufacture of e-liquids. A TRA should be carried out by a registered toxicological specialist and should include:
    - Hazard identification
    - Dose-response information
    - Exposure assessment
    - Risk characterisation
- All flavourings including natural extracts should be considered in the toxicological risk assessment of the product, where a TRA is deemed necessary.
- In case of tobacco extracts, additional analyses should be undertaken to ensure that tobacco-specific nitrosamines are measured and reduced to toxicologically supportable levels.
- If the substance or extract is no longer toxicologically supportable for use in e-liquid, it should be added to the list of substances stating which substances not to be added to or present in e-liquid. Each producer should maintain such a list, and where possible, these should be harmonised with other producers to maximise consumer protection.
- Preferably a centralised body should be established to coordinate harmonisation of a list of substances which should not be added to or present in e-liquids.

The PAS document contains the following **list of ingredients which should not be added to e-liquids**:

- Ingredients that are classified as CMR (it is noted that nicotine is not classified as CMR according to the CLP Regulation).
- Ingredients that are classified as respiratory sensitiser.
- Diacetyl or 2,3-butanedione (CAS 431-03-8), which has known inhalation risks and should not be used in flavourings. Producers should refer to the regulatory and scientific literature on permissible exposure levels.
- 2,3-pentendione (or acetyl propionyl) (CAS 600-14-6), which has known inhalation risks and should not be used in flavourings. Producers should refer to the regulatory and scientific literature on permissible exposure levels.
- Diethylene glycol (CAS 111-46-6) should not be added as ingredient, but might be present as contaminants in glycerol and propylene glycol. If present, the maximum level should be 0.1%.
- Ethylene glycol (CAS 107-21-1) should not be added as ingredient, but might be present as contaminants in glycerol and propylene glycol. If present, the maximum level should be 0.1%.
- Formaldehyde (CAS 50-00-0) should not be added as ingredient, but might be present. If present, it should not be present above toxicologically supportable levels (as identified by TRA).
- Acetaldehyde (CAS 75-07-0) should not be added as ingredient, but might be present. If present, it should not be present above toxicologically supportable levels (as identified by TRA).

- Acrolein (CAS 107-08-8) should not be added as ingredient, but might be present. If present, it should not be present above toxicologically supportable levels (as identified by TRA).
- Metals (Cd, Cr, Fe, Pb, Hg, Ni) should not be added to the e-liquids as ingredients but might be present. They should not be present above toxicological supportable levels (as identified by TRA).

Furthermore, the PAS document contains some recommendations regarding emissions from e-liquids, which are not described here, as the focus for this report is the ingredients in the e-liquids.

4.2 French standard XP D-90-300

The French standard XP D-90-300 consists of three parts dealing with requirements and test methods for:

- Part 1: Electronic cigarettes
- Part 2: E-liquids
- Part 3: Emissions

4.2.1 XP D 90-300 Part 1: Electronic cigarettes

Part 1 of the French standard (Afnor, 2015a) only deals with aspects concerning the e-cigarette and not the e-liquids. This part of the standard does not set requirements concerning chemical substances in e-liquids, but is limited to physical mechanical and thermal requirements of the e-cigarettes, and requirements for leakage of e-liquids from the e-cigarette. Furthermore, this part of the standard sets chemical requirement regarding the content of allergenic and toxic substances for the e-cigarette materials used for the mouthpiece, tank and coatings of the e-cigarette. This part of the standard is therefore not described further.

4.2.2 XP D 90-300 Part 2: E-liquids

Part 2 of the French standard (Afnor, 2015b) contains requirements for e-liquids and includes chemical requirements for the content of e-liquids. These chemical requirements are:

- Requirements concerning the purity of propylene glycol, glycerol and other diluents used.
- The nicotine used shall comply with the requirements of the European (Ph. Eur.) or American (USP) Pharmacopoeia.
- Flavouring compounds used shall be of food grade as defined in EU Regulation 1334/2008 on flavourings for food, and the flavouring compound may contain authorised food additives.
- Other ingredients shall comply with the requirements defined in EU Regulation 1333/2008 on food additives and EU Regulation 231/2012 concerning specifications for food additives, i.e. only approved additives for use in food as listed in Annex II and Annex III of the food additives Regulation 1333/2008 must be used.
- The following ingredients must not be used in e-liquids:
    - Substances classified as CMR (CMR 1 and 2)
    - Substances classified as STOT for the respiratory tract (STOT 1)
    - Oil or fat of plant or mineral origin (essential oils are not covered by this definition)

    - The following sugars: glucose, fructose, lactose, maltose, saccharose
    - The following sweeteners: acesulfame potassium, aspartame, sodium saccharinate, stevia
    - Vitamins and minerals
    - Pharmacologically active molecules (other than nicotine), i.e. medicinal, psychotropic, anabolic and narcotic substances, as well as stimulant additives such as caffeine or taurine
    - Preservatives liable to release formaldehyde
    - Triclosan (CAS 3380-34-5)
    - Phenoxyethanol (CAS 122-99-6)
    - Long-chain parabens, i.e. isopropylparaben (CAS 4191-73-5), isobutylparaben (CAS 4247-02-3), phenylparaben (CAS 17696-62-7), benzylparaben (CAS 94-18-8), pentylparaben (CAS 6521-29-5)
    - Isothiazolinone (CAS 1003-07-2)
    - Radioactive substances
    - Diacetyl (CAS 431-03-8)
    - Ehtylene glycol (CAS 107-21-1)
- If food allergens are used, they shall be specifically traced.
- The following limit values are listed for contaminants in e-liquids:
    - Diacetyl (22 mg/L)
    - Formaldehyde (22 mg/L)
    - Acrolein (22 mg/L)
    - Acetaldehyde (200 mg/L)
- The following limit values are listed for heavy metals in e-liquids, which are considered to be technically unavoidable trace levels:
    - Pb (10 mg/L)
    - As (3 mg/L)
    - Cd (1 mg/L)
    - Hg (1 mg/L)
    - Sb (5 mg/L)
- E-liquids must not constitute a microbiological risk.
- The information on the e-liquid product must contain a list of ingredients contained in the product in descending order of concentration. However, the flavouring compound does not need to be detailed.

4.2.3 XP D 90-300 Part 3: Emissions

Part 3 of the French standard (Afnor, 2016) only deals with emissions of the e-cigarettes and is therefore not described in detail here, as the focus of this report is the ingredients in the e-liquids[3].

This part 3 sets different requirements for the specific substances emitting from e-cigarettes, but also sets a requirement for a constant delivery of nicotine from the e-cigarettes. Finally, this standard sets requirements concerning the thermal risks in order to prevent the user to burn the lips or the mouth during use.

[3] However, in another report ("Requirements for substances formed or released during the evaporation of e-liquids used in electronic cigarettes") the focus will be on emissions and substances formed or released during the evaporation of e-liquids.

4.3 Summary and discussion regarding chemical requirements in standards for substances in e-liquids

Currently, no standards have been published by CEN/TC 437 "Electronic cigarettes and e-liquids", which was established in 2015. However, a British guidance document does exist, but is not to be considered as a standard. Furthermore, a French standard in three parts sets requirements for the physical appearance of the e-cigarettes (part 1), the e-liquids (part 2) and the emissions from the e-liquids (part 3).

Both the British guidance document (BSI, 2015) and the French standard (Afnor, 2015b) set some chemical requirements for substances contained in e-liquids. Both documents set requirements for the purity of the ingredients and include a list of ingredients, which are not allowed in e-liquids. Several specific substances on this list of forbidden ingredients are common for both the British guidance document and the French standard, but each document also contains specific substances not listed in the other document. The specific substances restricted by the two documents are listed in Table 1 below.

A restriction of substances classified as CMR is common for both documents, whereas the British guidance document restricts substances classified as respiratory sensitisers and the French standard restricts substances classified as STOT 1 for the respiratory tract. Furthermore, the British guidance document includes a common recommendation of performing a toxicological risk assessment (TRA) of the substances used in and emitted from e-liquids – only substances which are considered safe to be used when performing a toxicological risk assessment must be used.

Table 1: Overview of requirements to e-liquids in existing national standards

Chemical substance	CAS no.	Limit value	
		British PAS guidance document	**French Afnor standard (part 2)**
Diacetyl (2,3-butadione)	431-03-8	No use	22 mg/L
2,3-pentendione	600-14-6	No use	
Diethylene glycol	111-46-6	0.1%	
Ethylene glycol	107-21-1	0.1%	No use
Formaldehyde	50-00-0	No use, impurities below TRA value	22 mg/L
Acetaldehyde	75-07-0	No use, impurities below TRA value	200 mg/L
Acrolein	107-08-8	No use, impurities below TRA value	22 mg/L
Cd		No use, impurities below TRA value	1 mg/L
Cr		No use, impurities below TRA value	
Fe		No use, impurities below TRA value	
Pb		No use, impurities below TRA value	10 mg/L
Hg		No use, impurities below TRA value	1 mg/L
Ni		No use, impurities below TRA value	
As			3 mg/L
Sb			5 mg/L

Chemical substance	CAS no.	Limit value	
		British PAS guidance document	**French Afnor standard (part 2)**
Triclosan	3380-34-5		No use
Phenoxyethanol	122-99-6		No use
Long-chain parabens (isoproylparaben, isobutylparaben, phenylparaben, benzylparaben, pentylparaben)	4191-73-5 4247-02-3 17696-62-7 94-18-8 6521-29-5		No use
Isothiazolinone	1003-07-2		No use
CMR substances in general	-	All CMR: no use	CMR 1 and 2: no use
Respiratory sensitisers	-	No use	-
STOT 1 substances			No use
Oil or fat of plant or mineral origin			No use
Sugars (glucose, fructose, lactose, maltose, saccharose)			No use
Sweeteners (acesulfame potassium, aspartame, sodium saccharinate, stevia)			No use
Vitamins and minerals			No use
Pharmacologicall active molecules (other than nicotine)			No use
Radioactive substances			No use
Preservatives that release formaldehyde			No use
Toxic or dangerous substances in general		No use above the toxicological value as defined by TRA	

As described in the previous chapter on “Legislation”, neither the GPS Directive nor the Tobacco Directive contains specific requirements for e-cigarettes and e-liquids – both directives contain a general statement regarding ‘products must be safe’ or ‘ingredients must not pose a risk to human health’. It is therefore important that the standards developed set the necessary chemical requirements (for the necessary chemical substances contained in the e-liquids) and the correct limit values to protect the consumer from unwanted health effects from ingredients contained in e-liquids.

5 Screening of ingredients

In this chapter, a screening of chemical substances (ingredients) used in e-liquids has been performed by searching for articles on the subject on the internet.

5.1 Internet search for ingredients in e-liquids

The search has been carried out by searching for articles and reports on the subject, but also by searching for information on websites of internet shops selling e-liquids. However, the information on these sites is partial and was mostly limited to information about types of flavouring, e.g. 'orange' and not the actual chemical substances contained.

The articles are not described in detail, but have been used to create the table below, which is an overview of ingredients identified in e-liquids.

A total of 15 different articles was used as source and the underlying purpose, method of analysis, type of reported data etc. varies a lot between the articles. However, the majority of the articles have been published in scientific magazines. The oldest article used is from 2012, but most of the articles are not older than 2014.

The included ingredients are a long list, which does not distinguish between substances which were identified in few or several studies.

The total number of different ingredients found to be present in e-liquids is 109 (107 identified with CAS number). The approximate distribution of functions of the identified ingredients is:

- 4 solvents/diluents
- 64 substances in the aroma/flavour category
- 1 narcotic (nicotine)
- 2 pharma
- 23 pollutants/unintentionally added substances
- 15 substances with unknown function

In general, solvents make out the main ingredients in the e-liquids as regards to the concentration (% w/w) and the most common ones are propylene glycol, glycerin and ethylene glycol.

Nicotine is present in different quantities typically within the range of 6-24 mg per cartridge (Schraufnagel 2014). Several of the studied articles have also identified nicotine in a large number of samples claiming to be nicotine-free.

Pollutants/impurities in the liquids can be present for several different reasons, examples being derivatives/metabolites from the intentionally added ingredients or heavy metals originated from the heating elements or batteries in e-cigarettes (BMA, 2012). Pollutants are typically present in low concentrations.

It is likely that the majority of the ingredients for which the function is unknown fall within one of the categories aroma or pollutant.

It is expected that all ingredients present in the e-liquids also will be present in the vapour from the e-liquid, whereas not all substances found in the vapour will be present in the e-liquids. Table 2 contains ingredients identified as present in the e-liquids in this project.

5.2 Existing threshold values for ingredients

The purpose of this study is to develop a proposal for requirements for selected substances in e-liquids based on relevant existing threshold values for different chemical substances. It has therefore been examined if the identified ingredients in e-liquids are on different lists of existing threshold values for different chemical substances. The following schemes with existing threshold values have been examined and compared with the ingredients identified in e-liquids:

- EU-LCI Group
- The German AgBB scheme
- German indoor air guide values
- REACH DNEL values for registered substances
- ATSDR Minimal Risk Levels (MRLs)
- US EPA Reference Concentration (RfC) and Reference Dose (RfD) values
- WHO Air Quality Guidelines
- Other relevant reports for individual substances

5.2.1 EU-LCI Group

The EU LCI concept[4] was developed as part of a basic scheme for the evaluation of VOC emissions from building products. The objective of the European LCI Group is to elaborate and establish a common European list of chemicals and their associated toxicological thresholds relevant to human health. The relevant chemicals are identified via indoor air monitoring programs and emission testing of building products by EU countries.

The LCI values (Lowest Concentration of Interest) was originally based on either air quality guideline values (AQG) or occupational exposure limits, but a detailed framework for assessing chemicals emitted has now been developed, which involves a comprehensive data compilation (from sources like the REACH registration system, RIVM, Health Canada, US-EPA, WHO indoor air quality guidelines etc.) and data evaluation (JRC, 2013).

The EU-LCI Group defines EU-LCI in the following way:

- EU-LCIs are health-based values used to evaluate emissions after 28 days from a single product during a defined laboratory test chamber procedure.
- EU-LCIs are applied in product safety assessment with the ultimate goal to avoid health risks from long-term exposure for the general population.
- EU-LCI values are usually expressed as $\mu g/m^3$.
- Only values derived by application of the process established by the EU-LCI WG and described herein and ratified by the EU-LCI WG shall be called EU-LCIs.

The identified ingredients in e-liquid have been looked up in the EU-LCI Group list and the values are listed in Table 2. However, only a few of the identified

[4] https://ec.europa.eu/growth/sectors/construction/eu-lci/about_en

substances in e-liquids was found on the list of EU-LCI values (7 out of 107 substances). Most aromatic/flavouring substances are not found emitting from building products.

5.2.2 The German AgBB scheme

The German AgBB[5] (the Committee for Health-related evaluation of Building Products) scheme is a scheme for VOC emissions from construction products. The AgBB scheme was reviewed and edited the last time in February 2015.

The AgBB scheme (AgBB, 2015) consists of a health-related evaluation of emissions from building products based on substance specific values – the so-called LCI values (Lowest Concentration of Interest). The LCI values are derived by specialists using existing health-based evaluations of substances in the workplace as a starting point. The AgBB scheme takes amongst others the following values into consideration when establishing LCI values:

- Indicative and binding occupational exposure limit values set by the European Commission,
- National occupational exposure limits such as German, American and values applied in other EU member states,
- DNEL (derived no-effect level) values determined for inhalative occupational exposure or DNEL values determined for long-term inhalative consumer exposure under the REACH Regulation,
- Values or recommendations from the European Scientific Committee on Occupational Exposure Limits (SCOEL),
- And may also include in individual cases WHO indoor air quality guidelines.

It is written in the document which describes the AgBB scheme that the European LCI values (EU-LCI) are more elaborate and based on a comprehensible rationale. The AgBB scheme therefore adopts published EU-LCI values into the German LCI list. However, the AgBB scheme has LCI values for more substances compared to the EU-LCI list.

For carcinogenic substances, specific LCI values are only established if the carcinogenicity threshold is above the non-carcinogenic endpoints. Otherwise no LCI values are derived for carcinogenic properties according to EU categories 1A and 1B, as these substances are dealt with separately within the AgBB scheme: No carcinogen belonging to EU categories 1A and 1B may exceed a concentration of 0.01 mg/m³ after 3 days and the value of 0.001 mg/m³ after 28 days.

The German AgBB LCI list consists of about 190 different chemical substances in total (February 2015), compared to the EU-LCI values that exists for about 110 substances (December 2016). This means that the German AgBB LCI list (from 2015) has adopted the EU-LCI values existing in 2015, and LCI values are developed for other substances.

The identified ingredients in e-liquid have been looked up in the German AgBB LCI list and the values are listed in Table 2. The LCI values are usually measured in µg/m^3. However, only a few of the identified substances in e-liquids was found on the list of AgBB LCI values (8 out of 107 substances). Most aromatic/flavouring substances are not found emitting from building products.

[5] http://www.eco-institut.de/en/certifications-services/national-marks-of-conformity/agbb-scheme/

5.2.3 German indoor air guide values

The German Environment Agency (UBA – Umwelt Bundesamt) has established the German Committee on Indoor Guidelines, which has prepared indoor air guide values for 47 individual substances (UBA, 2016). Guide Value I and II have been established:

- Guide Value I represents the concentration of a substance in indoor air which is not expected to cause adverse health effects in sensitive persons even in the case of lifelong exposure (according to current knowledge and when considered individually).
- Guide Value II is an effect-related value based on toxicological and epidemiological knowledge of the effect threshold of a substance. Guide Value II is usually a long-term value. Guide Value II represents the concentration of a substance in indoor air at and above the value where action needs to be taken immediately because this concentration could endanger the health of sensitive persons including children, when they stay indoor constantly for long periods of time.

The guide values apply for individual substances and provide no indication of any possible combined effects with different substances. The guide values are given in mg/m^3 for the concentration of specific substances in indoor air. Guide Value I figures are in all cases lower than Guide Value II figures and are therefore used for the calculations.

A total of 5 of the substances identified in e-liquids was found on the German indoor air guide value list.

5.2.4 REACH DNEL values for registered substances

Toxicological as well as physical data delivered to ECHA in connection with a REACH registration is listed in the database of the REACH-registered substances. In this database DNEL (Derived No-Effect Level) values can be found for different exposures (oral, inhalation, dermal), for different populations (workers or consumers) and are frequency dependent (short term exposure or long-term exposure). A DNEL value is defined as an external exposure level below which an adverse effect on human health is not expected. This means that a lower DNEL values implies a more toxic substance.

The DNEL values in the database of REACH-registered substances are, however, derived by the companies who have registered the substances, and it may be difficult to evaluate the basis and the validity for these DNEL values.

The DNEL values for consumers and workers (long term exposure for systemic effects) have been looked up and are listed in Table 2 below, if the substances identified in e-liquids have been registered. Currently (March 2017), about 17,200 substances are listed in the ECHA database of registered substances. The DNEL values are measured in mg/m^3 for exposure via inhalation. It should be noted that half of the substances (51 out of 107 substances) was not registered and therefore no information on DNEL values was available. Furthermore, for some of the registered substances, no information on DNEL values was available (for 9 out of 107 substances) and for a couple of substances, the hazard was unknown (2 out of 107 substances). This means that all in all a DNEL value was identified for 47 of the 107 identified substances in e-liquids.

5.2.5 ATSDR Minimal Risk Levels

ATSDR (Agency for Toxic Substances & Disease Registry) is a federal public health agency of the U.S. Department of Health and Human Services. ATSDR has prepared Minimal Risk Levels (MRLs) for a number of prioritised substances that are considered to be hazardous for the human health or the environment. The MRLs are listed as "estimates, which are intended to serve as screening levels" for ATSDR health assessors and others in order to identify areas to investigate further[6].

The MRLs are derived by preparing a toxicological profile for the substances that includes an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. The MRLs are based on non-cancer health effects only and are not based on a consideration of cancer effects. ATSDR uses the no observed adverse effect level/uncertainty factor (NOAEL/UF) approach to derive the MRLs for hazardous substances. They are set below levels that, based on current information, might cause adverse health effects in the people most sensitive to such substance-induced effects. MRLs are derived for acute (1-14 days), intermediate (>14-364 days), and chronic (365 days and longer) exposure durations and for the oral and inhalation routes of exposure.

The MRL values for chronic exposure and for inhalation have been looked up for the substances identified as ingredients in e-liquids and are listed in Table 2 below. Furthermore, the MRL values for oral chronic exposure have been looked up. The used ATSDR MRL list is dated March 2016 and contains a total of 435 substances. Only 12 substances out of the 107 substances identified with CAS number as ingredients in e-liquids were listed on the ATSDR MRL list. The MRL values are measured in either mg/m^3 or ppm for the inhalation values or mg/kg bw/day for oral values.

5.2.6 US EPA Reference Concentration (RfC) and Reference Dose (RfD)

The US EPA (Environmental Protection Agency) has established the IRIS Program (Integrated Risk Information System) in order to protect the human health and the environment. Through the IRIS program, health hazards of chemicals found in the environment are identified and characterised. IRIS assessments provide the following toxicity values for health effects resulting from chronic exposure to chemicals[7]:

- Inhalation reference concentration (RfC), which is the concentration of a chemical that a person can inhale every day for a lifetime and that is not anticipated to cause harmful non-cancer health effects. The RfC can be compared to an exposure estimate concentration in mg/m^3.
- Oral reference dose (RfD), which is the amount of a chemical that a person can ingest every day for a lifetime and that is not anticipated to cause harmful non-cancer health effects. The RfD can be compared to an exposure estimate in mg/kg bw/day.

Currently (March 2017), the IRIS database[8] contains 511 substances. However, neither the RfC nor the RfD has been evaluated for all 511 substances. Both the RfC and the RfD have been looked up in the database for the substances identified to be present in e-liquids. For only 2 of the 107 substances identified in e-liquids, a

[6] https://www.atsdr.cdc.gov/mrls/index.asp
[7] https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#iniris
[8] https://cfpub.epa.gov/ncea/iris/search/index.cfm?keyword=101-48-4

RfC was identified. For 10 of the 107 substances, a RfD was identified. In all, either a RfC or RfD value was identified for 11 of the 107 substances. The values are listed in Table 2 below.

5.2.7 WHO Air Quality Guidelines

WHO (World Health Organisation) has established limit values for 35 chemical air pollutants based on a chemical risk assessment (WHO, 2000). For the organic pollutants, the WHO Guidelines have been used to establish limit values by both the EU-LCI Group and the German AgBB scheme (AgBB, 2015; JRC, 2013). However, the WHO Guidelines also set limit values for inorganic compounds (elements), which are not addressed in the mentioned schemes concentrating on construction products.

For substances with carcinogenic effects, the conclusion by WHO is most often that no safe threshold limit value can be set, but limit values (in ng/m^3) are then set for e.g. an excess life time risk of 1/10,000, 1/100,000 or 1/1,000,000 for a specific type of cancer. In these cases, the life time risk values of both 1/10,000 and 1/1,000,000 have been listed.

The WHO Air Quality Guidelines contain limit values for 7 of the 107 substances identified in e-liquids. These are only elements. Nicotine is mentioned, but no safe value was established and therefore no value has been listed for nicotine.

5.2.8 Other relevant reports for individual substances

For some of the individual substances identified to be used in e-liquids, a search has been made to find threshold limit values set for these substances. A general search was not performed for all substances identified, but for some of the substances used most often or in highest amounts.

For glycerine (glycerol) an OECD SIDS report was found (OECD, 2002). NOAEL values for inhalation concentrations are described as well as occupational exposure limit values for the substance.

NIOSH (the American National Institute for Occupational Safety and Health) has in 2016 prepared a document concerning occupational exposure to the following substances:

- 2,3-butanedione (diacetyl (DA))
- 2,3-pentanedione (acetyl propionyl (AP))

NIOSH has established so-called REL values (recommended exposure limits) for the substances as a time-weighted average (TWA) for up to 8 hours/day during a 40-hour week for a 45-year working life. The limit values are based on a lifetime occupational exposure and should have no more than 1/1000 excess risk of lung function falling below the lower limit of a normal lung function. The limit values given are listed in ppb, which have been calculated into the unit mg/m^3, by use of a converter using the molecular weight of the substance[9].

For benzophenone, a document from Michigan Department of Environmental Quality was found (Michigan DEQ, 2015). Here an initial risk screening level

[9] https://www.markes.com/Resources/Frequently-asked-questions/How-do-I-convert-units.aspx

(IRSL) was found based on a 2-year NTP study on rats and based on the conclusions made by IARC.

For linalool, an article on the safety assessment of linalool as a fragrance ingredient was found (Api et al., 2015). In this article, a NOAEC (No Observed Adverse Effect Concentration) is listed. This value is, however, higher as the DNEL value given in the ECHA database of registered substances.

For menthol an OECD SIDS report (OECD, 2003) has been prepared, but no threshold values regarding inhalation toxicity are given. Therefore, the DNEL value in the ECHA database of registered substances is used.

Table 2: Overview of ingredients identified in e-liquids
Only threshold values found are listed in the last right column "Threshold value". Substances which positively are also found in emissions of e-liquids from e-cigarettes are marked in **bold**. Substances selected for further review are marked with green shading.

Ingredient	**CAS no.**	**Function**	**Concentration (if mentioned)**	**Comment in literature**	**Reference/source**	**Threshold value (measured in mg/m^3)**
Acetamide	60-35-5	Unknown if content is intentional		Detected in 4 out of 28 samples, possible human carcinogen acc. to IARC 1999	Hutzler, 2014	*No information found*
Acetoin (3-Hydroxy-2-butanone)	513-86-0	?	Up to 529 µg/e-cigarette		Allen, 2015	*No information found*
Acetyl propionyl (AP) (2,3-pentanedione)	600-14-6	Aroma/flavour	20-432 µg/day (Farsalinos), Up to 64 µg/e-cigarette (Allen)	Found in 74.2% of 159 samples. Approved for food use, but associated with respiratory disease when inhaled. NIOSH limits for occupational exposure exist.	Farsalinos et al., 2015; Allen, 2015	NIOSH: 0.03808
Acetylpyrazine	22047-25-2	Aroma/flavour		In 3 out of 28 liquids	Hutzler, 2014	*No information found*
Amino-Tadalafil	385769-84-6	Derivate from Tadalafil		Found by testing	BfR, 2012; Pisinger et al., 2014; Cheng, 2014	*No information found*
Anabasine	40774-73-0	Impurity		Impurities of anabasine, myosmine or beta-nicotyrint found in a majority of the samples. A pyridine and piperidine alkaloid.	BMA, 2012; Cheng, 2014	*No information found*
Anatabine	581-49-7	Aroma/flavour		In 2 out of 28 liquids. Alkaloid.	Hutzler, 2014; Cheng, 2014	*No information found*
Anis alcohol	105-13-5	Aroma/flavour		Regulated by European Cosmetics Directive	Hutzler, 2014	REACH DNEL Inh. Cons.: 5.248; DNEL Inh. Work.: 21.284
Anisaldehyde	123-11-5	Aroma/flavour		In 3 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 4.35; DNEL Inh. Work.: 14.7
Anisaldehyde propylene glycol acetal	6414-32-0	Aroma/flavour		In 4 out of 28 liquids	Hutzler, 2014	*No information found*
Arsenic	7440-38-2	Impurity	Up to 35 ng/ml	77 of 183 samples above LOQ. Median level below LOQ.	Visser, 2015	WHO: 0.000066 (if risk: 1/10,000) 0.00000066 (if risk: 1/1,000,000)

Ingredient	CAS no.	Function	Concentration (if mentioned)	Comment in literature	Reference/source	Threshold value (measured in mg/m^3)
Benzaldehyde	100-52-7	Aroma/flavour	Up to 21.2 mg/mL	In 4 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 4.9; DNEL Inh. Work.: 9.8; AgBB LCI: 0.09; German Indoor Air, Guide Value I: 0.02
Benzaldehyde propylene glycol acetal	2568-25-4	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Benzoic acid	65-85-0	?			Shraufnagel, 2014	REACH DNEL Inh. Cons.: 1.5; DNEL Inh. Work.: 3
Benzophenone	119-61-9	Aroma/flavour		In 3 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 0.17; DNEL Inh. Work.: 0.7; Other info: 0.000152
Benzyl acetate	140-11-4	Aroma/flavour		In 3 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 5.5; DNEL Inh. Work.: 21.9
Benzyl alcohol	100-51-6	Aroma/flavour		In 3 out of 28 liquids, Regulated by European Cosmetics Directive	Hutzler, 2014	REACH DNEL Inh. Cons.: 5.4; DNEL Inh. Work.: 22; EU-LCI: 0.440; AgBB LCI: 0.440; German Indoor Air, Guide Value I: 0.4
Benzyl benzoate	120-51-4	Aroma/flavour		In 4 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 1.25; DNEL Inh. Work.: 5.1
Butyl acetate	123-86-4	?			Shraufnagel, 2014	REACH DNEL Inh. Cons.: 35.7; DNEL Inh. Work.: 300; EU-LCI: 4.8; AgBB LCI: 4.8
Butyl carbitol	112-34-5	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 40.5; DNEL Inh. Work.: 67.5; EU-LCI: 0.67; AgBB LCI: 0.67 German Indoor Air, Guide Value I: 0.4
Cadmium	**7440-43-9**	**Impurity**	**Up to 81 ng/ml**	**Only 6 of 183 samples above LOQ (1 ng/ml)**	**Visser, 2015**	**REACH DNEL Inh. Work.: 0.004; ATSDR – MRL inhalation: 0.00001; WHO: 0.000005**
trans-Carane	18969-23-5	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Carvone	6485-40-1	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 0.289; DNEL Inh. Work.: 1175
4-Chloro-2,5-	6358-64-1	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*

Ingredient	CAS no.	Function	Concentration (if mentioned)	Comment in literature	Reference/source	Threshold value (measured in mg/m^3)
dimethoxy-aniline						
Chromium (elemental chromium)	**7440-47-3**	**Impurity**	**Up to 2.243 µg/ml**	**77 of 183 samples above LOQ. Median level below LOQ.**	**Visser, 2015**	**REACH DNEL Inh. Cons.: 0.027; DNEL Inh. Work.: 0.5; WHO: based on Cr(VI): 0.0000025 (if risk: 1/10,000) 0.000000025 (if risk 1/1,000,000)**
1,8-Cineol	470-82-6	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 1.74; DNEL Inh. Work.: 7.05
Cinnamial aldehyde	104-55-2	Aroma/flavour		In 2 out of 28 liquids, is an allergen	Hutzler, 2014	REACH DNEL Inh. Cons.: 2605; DNEL Inh. Work.: 21878
Citral	5392-40-5	Aroma/flavour		In 5 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 2.7; DNEL Inh. Work.: 9
Cobalt	7440-48-4	Impurity	Up to 482 ng/ml	Only 8 of 183 samples above LOQ	Visser, 2015	REACH DNEL Inh. Cons.: 0.0063 (local effects); DNEL Inh. Work.: 0.040 (local effects); ATSDR – MRL inhalation: 0.0001
Copper	7440-50-8	Impurity	Up to 45.54 µg/ml	80 of 183 samples above LOQ. Median level below LOQ.	Visser, 2015	REACH DNEL Inh. Work.: 20
Corylon	80-71-7	Aroma/flavour		In 5 out of 28 liquids	Hutzler, 2014	*No information found*
Cotinine	486-56-6	Impurity	Up to 178 µg/mL	Alkaloid.	Cheng, 2014.	*No information found*
Coumarin	91-64-5	Additive/flavour		Detected in 4 of 28 samples. Prohibited in tobacco in Germany	Hutzler, 2014	REACH DNEL Inh. Cons.: 0.183; DNEL Inh. Work.: 0.741
α-Damascenone	57549-92-5	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Damascenone (α or β)	23696-85-7	Aroma/flavour		In 7 out of 28 liquids	Hutzler, 2014	*No information found*
n-Decanoic acid	334-48-5	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Diacetin	25395-31-7	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*

Ingredient	CAS no.	Function	Concentration (if mentioned)	Comment in literature	Reference/source	Threshold value (measured in mg/m^3)
Diacetyl (DA)	431-03-8	Aroma/flavour	26-278 µg/day (Farsalinos), up to 239 µg/e-cigarette	Found in >74.2% of 159 samples in one study and in 39 of 51 samples in another. Approved for food use, but associated with respiratory disease when inhaled. NIOSH limits for occupational exposure exist. Is restricted by the Afnor standard.	Shraufnagel, 2014; Afnor, 2015b	NIOSH: 0.01761
1,1-Diethoxy ethane	105-57-7	Solvent	Up to 40 µg/g		Varlet, 2015	*No information found*
(2,2-Diethoxyethyl)-benzene	101-48-4	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Diethylenglycol	111-46-6	Humectant or pollutant	1%	Found in one product. Is a humectant for tobacco acc. to wikipedia.	BfR, 2012; Pisinger et al., 2014; BMA, 2012; Shraufnagel, 2014	REACH DNEL Inh. Cons.: 12; DNEL Inh. Work.: 44; EU-LCI: 5.7; AgBB LCI: 0.44
Diisobutyl phthalate	84-69-5	Aroma/flavour		In 3 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 0.72; DNEL Inh. Work.: 2.94
p-Dimethoxybenzene	150-78-7	?	Up to 15.6 mg/l		Schober, 2014	*No information found*
2,6-dimethyl phenol	576-26-1	?			Shraufnagel, 2014	REACH DNEL Inh. Work.: 2
Dioctyl carbonate	105-58-8	?			Shraufnagel, 2014	REACH DNEL Inh. Cons.: low hazard (no threshold derived); DNEL Inh. Work.: 0.2
Dioctyl phthalate	117-84-0	?			Shraufnagel, 2014	*Only oral values identified*
γ -Dodecalactone	2305-05-07	?	Up to 0.6 mg/l		Schober, 2014	*No information found*
Ethyl acetate	141-78-6	Aroma/flavour	Up to 7.1 mg/mL		Tierny et al., 2015; Varlet 2015	REACH DNEL Inh. Cons.: 367; DNEL Inh. Work.: 734; AgBB LCI: VVOC German Indoor Air, Guide Value I: 0.6
Ethyl butyrate	105-54-4	Aroma/flavour	Up to 11.1 mg/mL		Tierny et al., 2015	*No information found*
Ethyl maltol (2-Ethyl-3-hydroxy-4-pyranone)	4940-11-8	Aroma/flavour	Up to 23.4 mg/mL	In 16 out of 28 liquids	Hutzler, 2014; Tierny et al., 2015	*No information found*

Ingredient	CAS no.	Function	Concentration (if mentioned)	Comment in literature	Reference/source	Threshold value (measured in mg/m^3)
Ethyl mandelate	774-40-3	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Ethyl phenylacetate	101-97-3	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Ethyl vanillin	121-32-4	Aroma/flavour	Up to 8.4 mg/mL	In 14 out of 28 liquids	Hutzler, 2014; Tierny et al., 2015	*No information found*
Ethylene glycol	107-21-1	Solvent	1-76% (av=26%)	Replaced PG in five products. Is restricted by the Afnor standard.	Hutzler, 2014; Afnor, 2015b	REACH DNEL Inh. Cons.: 7 (local effects); DNEL Inh. Work.: 35; EU-LCI: 3.4; AgBB LCI: 0.26; ATSDR – MRL inhalation: 2.0 (acute)
Eugenol	97-53-0	Aroma/flavour		In 5 out of 28 liquids, Regulated by European Cosmetics Directive	Hutzler, 2014	REACH DNEL Inh. Cons.: 5.22; DNEL Inh. Work.: 21.2
Furaneol	3658-77-3	?	Up to 7622.7 mg/l		Schober, 2014	*No information found*
Glycerine (Glycerol)	**56-81-5**	**Solvent (sometimes replace PG)**	**7-42% (average = 26%)**		**BfR, 2012; Hutzler, 2014**	**REACH DNEL Inh. Cons.: 33 (local effects); DNEL Inh. Work.: 56; OECD SIDS: 10**
γ-Heptalactone	105-21-5	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
1,2-Hexanediol	629-11-8	Aroma/flavour		In 3 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 8.7; DNEL Inh. Work.: 35
Hydrocoumarine	119-84-6	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Isoamyl butylate	106-27-4	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Isobornyl acetate	125-12-2	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 13.04; DNEL Inh. Work.: 13.22
Lead	**7439-92-1**	**Impurity**	**Up to 4.931 µg/ml**	**Only 16 of 183 samples above LOQ (5 ng/ml)**	**Visser, 2015**	**WHO: 0.0005**
Limonene	138-86-3	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	EU-LCI: 5.0; AgBB LCI: 5.0 German Indoor Air, Guide Value I: 1
Linalool	78-70-6	Aroma/flavour		In 6 out of 28 liquids, common ingredient in perfumes, Regulated by European Cosmetics Directive	BfR, 2012; Hutzler, 2014	REACH DNEL Inh. Cons.: 0.7; DNEL Inh. Work.: 2.8; Other info: 63
Maltol	118-71-8	Aroma/flavour	Up to 6.2 mg/mL		Tierny et al., 2015	*No information found*
Manganese	7439-96-5	Impurity	Up to 7.613 µg/ml	71 of 183 samples above LOQ.	Visser, 2015	REACH DNEL Inh. Cons.: 0.041;

Ingredient	CAS no.	Function	Concentration (if mentioned)	Comment in literature	Reference/source	Threshold value (measured in mg/m^3)
				Median level below LOQ.		DNEL Inh. Work.: 0.2; ATSDR – MRL inhalation: 0.00003; US EPA RfC: 0.14; WHO: 0.00015
p-Menthane-1,2-diol	336669-76-0	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Menthol	89-78-1	Aroma/flavour	Up to 21.6 mg/mL	In 12 out of 28 liquids	BfR, 2012; Hutzler, 2014; Tierny et al., 2015	REACH DNEL Inh. Cons.: 16.3; DNEL Inh. Work.: 66.28
Methyl cinnamate	103-26-4	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 6.96; DNEL Inh. Work.: 28.8
Methyl dihydrojasmonate	14851-98-7	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
3-Methyl-1,2-cyclopentanedione	765-70-8	Aroma/flavour		In 6 out of 28 liquids	Hutzler, 2014	*No information found*
4-Methyl-2-pentyl-1,3-dioxolane	1599-49-1	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Miosmine (or myosmine)	532-12-7	Aroma/flavour		In 2 out of 28 liquids(Arch), Impurities of anabasine, myosmine or beta-nicotyrint found in a majorith of the samples (BMA). An alkaloid.	Hutzler, 2014; BMA, 2012	*No information found*
Molybdenum	7439-98-7	Impurity	Up to 53 ng/ml	Only 16 of 183 samples above LOQ	Visser, 2015	REACH DNEL Inh. Cons.: 3.33; DNEL Inh. Work.: 11.7
Myosmine	536-12-7	Impurity	Up to 71 µg/mL	Alkaloid.	Cheng, 2014.	*No information found*
Nickel	**7440-02-0**	**Impurity**	**Up to 225.9 µg/ml**	**Only 27 of 183 samples above LOQ (10 ng/ml)**	**Visser, 2015**	**REACH DNEL Inh. Cons.: 0.020; DNEL Inh. Work.: 0.05; ATSDR – MRL inhalation: 0.00009; WHO: 0.00025 (if risk: 1/10,000) 0.0000025 (if risk: 1/1,000,000)**
Nicotine	54-11-5	Psychedelic	Most contain between 6-24 mg, but some more than 100 mg per cartridge (FIRS, 2014).	Addictive. Extreme use of e-cigarettes has led to acute nicotine poisoning. LD50 < 5 mg/kg bw. BfR reference dose = 0.0008 mg/kg bw.	Cheng, 2014.	*No information found* WHO: no safe value
Nicotine-N-oxide	??	Impurity	0,03 -0,16% of nicotine content	Alkaloid.	Multiple	*No information found*

Ingredient	CAS no.	Function	Concentration (if mentioned)	Comment in literature	Reference/source	Threshold value (measured in mg/m^3)
β-nicotyrine	487-19-4	Impurity	Up to 6 µg/mL (Chen)	Impurities of anabasine, myosmine or beta-nicotyrint found in a majority of the samples. Alkaloid metabolite of nicotine	BMA, 2012; Cheng, 2014.	*No information found*
N-nitrosoanabasine (NAB)	37620-20-5	Impurity	0.11 – 11.11 µg/L and up to 0.69 ng/cartridge in other study (Cheng), 0.9 ng/16mg cartridge (Orr)	Tabacco-specific nitrosamine	Cheng, 2014; Orr, 2014.	*No information found*
N-nitrosoanatabine (NAT)	71267-22-6	Impurity	0.09 – 62.19 µg/L and up to 2.16 ng/cartridge in other study (Cheng), 2.16 ng/16mg cartridge (Orr.)	Tabacco-specific nitrosamine	Cheng, 2014; Orr, 2014.	*No information found*
N-nitrosonornicotine (NNN)	**16543-55-8**	**Impurity**	**0.34 – 60.08 µg/L and up to 3.87 ng/cartridge in other studies (Cheng), up to 0.00043 µg/ 15 puffs (Drummond), 3.87 ng/16mg cartridge (Orr)**	**Tabacco-specific nitrosamine. IARC classification - Group 1 carcinogen.**	**Cheng, 2014; Drummond et al., 2014; Orr, 2014.**	***No information found***
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (Nitrosamine ketone - NNK)	64091-91-4	Impurity	0.22 – 9.84 µg/L and up to 1.46 ng/cartridge in other studies (Cheng), Up to 0.00283 µg/ 15 puffs (Drummond), 1.46 ng/16mg cartridge (Orr)	Tabacco-specific nitrosamine	Cheng, 2014; Drummond et al., 2014; Orr, 2014.	*No information found*
γ-Nonalactone	104-61-0	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 3.97; DNEL Inh. Work.: 16.1
γ-Octalactone	104-50-7	Aroma/flavour		In 4 out of 28 liquids	Hutzler, 2014	*No information found*
Phenolethyl alcohol	60-12-8	Aroma/flavour		In 3 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 17.7;

Ingredient	CAS no.	Function	Concentration (if mentioned)	Comment in literature	Reference/source	Threshold value (measured in mg/m^3)
						DNEL Inh. Work.: 59.9
Piperitone	89-81-6	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Piperonal	120-57-0	Aroma/flavour		In 7 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 4.3; DNEL Inh. Work.: 17.6
Piperonal propyleneglycol acetal	61683-99-6	Aroma/flavour		In 5 out of 28 liquids	Hutzler, 2014	*No information found*
p-Propenylansiole	104-46-1	?	Up to 13.4 mg/l		Schober, 2014	*No information found*
Propionaldehyde	**123-38-6**	**?**		**Significant amounts were only found at 150° C by GC-MS analysis**	**Hutzler, 2014**	**REACH DNEL Inh. Work.: 6.1; AgBB LCI: VVOC; US EPA RfC: 0.008**
Propylene glycol (1,2-Propandiol, PG)	**57-55-6**	**Solvent**	**2-79% (average = 53%)**	**A main ingredient. More details about limit values in BfR.**	**BfR, 2012; Hutzler, 2014; Tierny et al., 2015; Hahn 2014**	**REACH DNEL Inh. Cons.: 50; DNEL Inh. Work.: 168; EU-LCI: 2.1; AgBB LCI: 2.5; ATSDR – MRL inhalation: 0.02801 (0.009 ppm)**
Pulegone	89-82-7	Aroma/flavour		In 3 out of 28 liquids	Hutzler, 2014	*No information found*
Quinoline	91-22-5	?			Shraufnagel, 2014	REACH DNEL Inh. Cons.: low hazard (no threshold derived); DNEL Inh. Work.: 0.005
Rheosmin (Raspberry ketone)	5471-51-2	Aroma/flavour		In 1 out of 28 liquids	Hutzler, 2014	REACH DNEL Inh. Cons.: 59.5; DNEL Inh. Work.: 114.24
Rimonabant	158681-13-1	Pharma			BfR, 2012; Pisinger et al., 2014; Cheng, 2014	*No information found*
Syringol	91-10-1	Aroma/flavour		In 2 out of 28 liquids	Hutzler, 2014	*No information found*
Tabacco-specific nitrosamine (TSNA)	-	Pollutant	Trace level (BfR), 8,18 ug/g in cartridge (Orr)	Includes nicotine and several related compounds and their metabolites incl. NNN, NAB, NAT, NNK addressed individually in the table.	BfR, 2012; BMA, 2012; Cheng, 2014; Orr, 2014	*No information found*
Tadalafil	171596-29-5	Pharma			BfR, 2012; Pisinger et al., 2014	*No information found*
Terpineol	7785-53-7	Aroma/flavour		In 5 out of 28 liquids	Hutzler, 2014	*No information found*

Ingredient	**CAS no.**	**Function**	**Concentration (if mentioned)**	**Comment in literature**	**Reference/source**	**Threshold value (measured in mg/m^3)**
Thujone	76231-76-0	Aroma/flavour	6.7 mg/l on average.	Detected in 4% of studied samples. $BMDL_{10}$ = 11 tested on clonic seizures on rats	Hahn, 2014	*No information found*
Tin	**7440-31-5**	**Impurity**	**Up to 2,000 µg/ml**		**Visser, 2015; Pisinger et al., 2014**	**REACH DNEL Inh. Cons.: 17; DNEL Inh. Work.: 71**
p-Tolualdehyde	14-87-0	Aroma/flavour	Up to 2.8 mg/mL		Tierny et al., 2015	*No information found*
Trimethylpyrazine	14667-55-1	Aroma/flavour		In 6 out of 28 liquids	Hutzler, 2014	*No information found*
Uranium	7440-61-1	Impurity	Up to 2.2 ng/ml	Only 2 of 183 samples above LOQ	Visser, 2015	ATSDR – MRL inhalation: 0.00004
Vanadium	7440-62-2	Impurity	Up to 60 ng/ml	Only 6 of 183 samples above LOQ	Visser, 2015	REACH DNEL Inh. Cons.: 6460 (no hazard identified); DNEL Inh. Work.: 13098 (no hazard identified); ATSDR – MRL inhalation: 0.0001; WHO: 0.001
Vanillin (4-hydroxy-3-methoxybenzaldehyd)	121-33-5	Aroma/flavour	Up to 33 mg/mL	In 22 out of 28 liquids(arch) and 17 out of 30 samples (Tierney)	Hutzler, 2014; Tierny et al., 2015	*No information found*
Zinc	7440-66-6	Impurity	Up to 55,295 µg/ml	155 of 183 samples above LOQ. Median value were 55 ng/ml.	Visser, 2015	REACH DNEL Inh. Cons.: 2.5; DNEL Inh. Work.: 5

6 Selection of ingredients

In the selection of ingredients, the following criteria have been used:

- Substances which according to the references seem to be used in the highest concentrations in the e-liquids will be prioritised first.
- Substances which according to the references seem to be used in several e-liquids will be prioritised first.
- Substances with the lowest threshold values have been selected.
- Mainly identified substances in e-liquids with an identified threshold value have been selected for further review.
- Substances with different functions in e-liquids have been selected.

A total of 15 ingredients were selected for further review. These have been marked with green shading in Table 2 above, and these are:

1. Glycerine (glycerol)
2. Propylene glycol (1,2-Propandiol, PG)
3. Ethylene glycol
4. Diacetyl (DA)
5. Acetyl propionyl (AP)
6. Lead
7. Cadmium
8. Nickel
9. Benzophenone
10. Coumarin
11. Carvone
12. Linalool
13. Benzaldehyde
14. Benzyl alcohol
15. Menthol

Most substances selected are aroma/flavourings, 3 substances are elements and 3 substances selected are solvents.

Nicotine is not amongst the selected substances, as a limit value already exists in the legislation for nicotine.

Glycerine, propylene glycol and ethylene glycol have been chosen for further review as these are the substances present in the highest concentration in the e-liquids. Likewise, the substances diacetyl (DA) and acetyl propionyl (AP) are selected, as these two substances seem to be used in many e-liquids (in about 70% according to two studies). Furthermore, the elements lead, cadmium and nickel are selected, as these elements are the most toxic elements (Pb and Cd) and are restricted in many other consumer products as well. Finally, menthol has been chosen because it seems to be used in many e-liquids, and the rest of the substances are selected as they are the substances with the lowest threshold values, i.e. the most toxic substances.

7 Exposure calculation

For the selected ingredients in e-liquids (see chapter 6), a proposal for limit values in the e-liquids is calculated. The calculation is based on the identified existing threshold limit values for the selected substances.

The proposals for limit values in the e-cigarette liquid are calculated as a concentration of the ingredient in the e-liquid and are based on assumptions and worst-case scenarios regarding e-cigarette use. However, it should be emphasised that even though the term 'worst case scenarios' is used, this does not guarantee that a few individuals in extreme cases will be exposed to larger amounts than used in the 'worst case scenarios'.

In this chapter, the calculation method is described and the worst-case values used are described and discussed.

Existing threshold limit values in the form of e.g. ECHA DNEL values and US Reference Concentration or Dose values are assumed to be derived in a similar way by use of appropriate safety and uncertainty factors. In practice, the differences between values may occur as a function of the size of the safety and uncertainty factors used, and perhaps also as a function of the date of establishing the threshold value, as the most recent values may be based on new data on toxicity. The difference between the values is, however, that ECHA DNEL values are calculated by industry and based on registrations to ECHA by companies producing these chemicals whereas Reference Concentration or Dose values are prepared by independent scientists (toxicologists). For this reason, the ECHA DNEL values are only used if other values are not available.

In some situations, only threshold limit values set for indoor air or construction products are available. In these situations, these limit values are used.

One important aspect of this report is that typically only the long-term (or intermediate-term) local or systemic effects of the inhaled substances are taken into considerations. However, in some cases available limits (e.g. occupational or indoor-related limits) were used without knowing the rationale behind these limits.

7.1 Calculation method

The core information to be established is which concentration of a substance in an e-liquid can be assumed to be safe for a person with a given daily consumption of e-liquid ("*the safe concentration"*), using a conservative approach. This concentration is calculated by use of conservative assumptions regarding daily intake of e-liquid. The basic unit for this *safe concentration* will be mg/ml (or µg/ml) daily consumption of e-liquid.

This *safe concentration* in the e-liquid is calculated on the basis of information about the *safe daily dose or safe daily total amount of a substance*, which is possible to inhale or take in the body each day without the risk of long-term health effects.

It has been decided for this report only to use threshold values (given in mg/m^3) based on inhalation (in contrast to threshold limit values given in mg/kg bw/day, which are based on intake of the substance) – simply because the exposure pathway for e-liquids is by inhalation.

The *safe daily dose or safe daily total amount of a substance* can be established from DNEL values or Reference Concentration values or other threshold limit values in mg/m^3, where the DNEL/RfC value is multiplied by the assumed daily (i.e. 24 hours) volume of inhaled air. However, if a threshold limit value in the working environment is used, the value should be multiplied by the volume of air inhaled during a working day, i.e. 8 hours. This method is based on the assumptions that all the inhaled e-liquid will be absorbed in the body and that all vapour from the e-cigarette is inhaled.

Regarding the consumption/inhalation of e-liquid per day, it is important to notice that this daily consumption strongly will depend on the nicotine level in the e-liquid (from no nicotine to highest allowed nicotine level (20 mg/ml)). Several discussion forums can be found debating the typical consumption of e-liquids per day[10]. It is therefore important that the proposed limit values for substances in e-liquids will be valid for all e-liquids irrespective of nicotine content (from 0 to 20 mg/ml). As a worst case, a consumer may "chain smoke" on an e-cigarette with a low or no content of nicotine, i.e. getting a low or no level of nicotine, but high level of other ingredients.

The formula below shows how to calculate the maximum concentration of a substance in an e-liquid by use of the *safe dose or daily total amount of a substance* given in mg/m^3:

$$C_{Substance\_in\_e\_liquid} = \frac{V_{air\_daily} \times RfC_{substance}}{abs_{substance} \times V_{Liquid\_per\_puff} \times n_{puffs}}$$

$C_{Substance\_in\_e\_liquid}$	Is the maximum concentration of the substance in the e-liquid (mg/ml) that will not result in adverse effects, i.e. the limit value that should be set for the substance
V_{air_daily}	Is the volume of air inhaled per day (24 hours) or per 8 hours where occupational limits were used (m^3)
$RfC_{substance}$	Is the DNEL or Reference Concentration (existing threshold limit values available) for the substance (mg/m^3)
$abs_{substance}$	Is the absorption coefficient (%) of the substance, i.e. how large a part of the substance that will be absorbed in the body, when inhaling e-cigarette vapour. It is assumed that by default in this screening project 100% of the substance will be absorbed in the body (worst case). By default this absorption coefficient will then be 1.
$V_{Liquid\_per\_puff}$	Is the volume of e-liquid used (vaporised) per puff (ml/puff)
n_{puffs}	Is the total number of puffs per day (puffs/day)

[10] https://www.e-cigarette-forum.com/forum/threads/average-daily-e-liquid-consumption.414565/; https://www.nicvape.com/About-Nicotine-Strengths; https://www.planetofthevapes.co.uk/forums/ecig-discussion/general-chat/threads/common-misunderstanding-1-ml-liquid-can-not-be-compared-with-a-cigarette.52078/

7.2 Assumptions used

The following assumptions are made in the screening calculations:

- The entire amount of a substance in the e-liquids is assumed to be vaporised.
- All substances in the e-liquids are vaporised without being chemically changed or without undergoing a chemical reaction during the vaporisation process.
- The entire amount of the substance being vaporised for each puff is assumed to be inhaled by the user.
- Each puff contains the same quantity of a substance in the e-liquid, i.e. it is assumed that the e-cigarette will deliver a constant concentration of substances for each puff.
- The entire amount of the substance being inhaled is assumed to be absorbed in the human body, which means that $abs_{substance} = 1$.
- Typically, long term systemic effects are taken into considerations in this report. In some cases, a different approach may be used, where only data for acute effects such as irritation or other acute effects were available.

The following values are used in the calculations:

$abs_{substance}$
As described above it is assumed that the entire amount of the substance being inhaled will be absorbed in the body. This means that a factor of 1 is used for $abs_{substance}$.

V_{air_daily}
As a worst-case value for daily inhaled volume of air (also called inhalation rate), the value of 16 m^3 is used. ECHAs "Guidance document for consumer exposure" (ECHA, 2016) refers to among others the US EPA "Exposure Factors Handbook" (US EPA, 2011) and RIVM "General default parameters for estimating consumer exposure" (RIVM, 2014) for default values on inhalation rates. However, in an earlier ECHA Guidance document (ECHA, 2012) a default inhalation of 18 m^3 was suggested for long term daily inhalation rate for adults (age 20-75).

According to the US EPA (2011), the inhalation rate differs across age and gender. Adults have a higher inhalation rate compared to children, young adults have a higher inhalation rate compared to average adults, and males have a higher inhalation rate compared to females. The male daily inhalation rate lies between 19.3 m^3 for 16 to <21 year olds and 16.5 m^3 for > 60 year olds. The same value for females is 14.6 m^3 and 12.9 m^3. The daily inhalation rate for a weighted combination of gender has values between 17.0 and 14.7 m^3 across the different age groups.

According to RIVM (2014), the long-term inhalation rate for adults is 16 m^3 (based on Human Exposure Expert Group opinion (2013)) or 18 m^3 (based on ECHA (2012) value).

For this reason, a value of 16 m^3 is used as the worst-case value (the lowest value will result in the lowest *safe dose* and thereby the lowest calculated limit value in the e-liquids).

In the cases where the threshold limit values used are based on occupational limit values, i.e. an 8-hour working day, the inhaled volume will be one third of an entire day's (24 hours) inhaled volume, corresponding to 5.33 m^3.

$V_{Liquid\ per\ puff}$
This value is the volume of e-liquid being used (being vaporised) per puff. The value used by BfR (2015) of 150 puffs per ml e-liquid is also used in the calculations in this report. This means that one puff equals 1/150 = 0.0067 ml of e-liquid.

Other non-scientific references state that each drop of e-liquid (20 drops equals 1 ml) will last for approximately 7 puffs, which means that 1 ml e-liquid is approximately 140 puffs[11].

n_{puffs}
Of course, the number of puffs per day depends on how heavy an e-cigarette smoker the individual smoker is. Several studies[12] indicate that the number of puffs per day also is dependent on the concentration of nicotine in the e-liquid, i.e. if the nicotine concentration is too low, the e-cigarette smoker will typically puff more on the e-cigarette during the day to get the amount of nicotine wanted/needed – or in the case of e-liquids without nicotine, the consumer may assume that it is safe and will therefore not limit their smoking on non-nicotine containing e-cigarettes.

RIVM (2015) has in a report on the health risk of e-cigarettes made a questionnaire for a representative group of 456 daily or weekly users of e-cigarettes in the Netherlands. Based on this study they divide the e-cigarette smokers into the following categories:

- Light vapour: 15 inhalations (puffs) per day
- Average vapour: 60 inhalations (puffs) per day
- Heavy vapour: 500 inhalations (puffs) per day

The light vapour category mentioned in RIVM (2015) could be vapours only vaping a couple of times per week (i.e. weekly users and not daily users) or vapours just vaping once a day.

Other studies/articles use an average of 150 puffs per day (e.g. Burstyn (2014) and BfR (2015)), but as high as 700 puffs per day have been reported. As an example, a debate forum varies between about 200 puffs (= 1.5 ml e-liquid) and > 400 puffs per day (with different nicotine levels)[13], but people have reported up to 10 ml (=1500 puffs) per day, and many seem to use about 3-5 ml e-liquid per day.

In this project, the RIVM value of 500 puffs per day is used as a high value – however, it is not considered to be a worst case value, as different chat forums illustrate that even higher puffs per day are used by extremely heavy vapours. According to the values used by BfR (2015), 500 puffs per day would equal a consumption of 3.3 ml e-liquid per day. It should be emphasised that in extreme cases the calculated limit values (in chapter 8) still may not be sufficient because more heavy vapours do exist.

[11] https://www.e-cigarette-forum.com/forum/threads/how-many-hits-equal-smoking-a-cigarette.244923/
[12] https://www.e-cigarette-forum.com/forum/threads/average-daily-e-liquid-consumption.414565/; https://www.nicvape.com/About-Nicotine-Strengths; https://www.planetofthevapes.co.uk/forums/ecig-discussion/general-chat/threads/common-misunderstanding-1-ml-liquid-can-not-be-compared-with-a-cigarette.52078/
[13] https://www.e-cigarette-forum.com/forum/threads/how-many-puffs-a-day-do-you-take.388452/

In the Afnor standard, the limit values are set per 200 puffs. For this reason, the limit value for 200 puffs is calculated as well.

To summarise the following values are used in the calculations:

$abs_{substance}$	1
V_{air_daily}	16 m^3/day, because this value is used in exposure calculations by ECHA. However, when the used threshold values are based on an 8-hour working day, the inhaled volume will be one third of an entire day's inhaled volume, corresponding to 5.33 m^3.
$V_{Liquid\_per\_puff}$	150 puffs equal 1 mL, because this value seems to be used in several sources.
n_{puffs}	500 puffs per day, because this number represents a "heavy" e-cigarette smoker. However, the calculations based on 200 puffs per day are performed as well, as this unit is used in e.g. the Afnor standard.

8 Preliminary proposal for limit values of selected ingredients

In this chapter calculations for a preliminary proposal for limit values have been made for 15 selected ingredients. The limit values are considered as a preliminary proposal considering the limitations in this screening project. The limitations are discussed in chapter 9 "Discussion and recommendations".

The threshold limit values (TLVs) identified and their references used for the calculations are presented in Table 3 below. In general, the lowest threshold limit values have been used in the calculations of the proposed limit values of the selected ingredients. However, this general rule has been deviated for a few substances where e.g. newer data seems more valid or appropriate:

- For propylene glycol, an ATSDR MRL inhalation value of 0.02801 mg/m^3 is listed, where EU-LCI and AgBB LCI values are 2.1 and 2.5 mg/m^3 respectively. The MRL value was adopted in 1997 and uses a high uncertainty factor of 1000, whereas the EU-LCI values were adopted in 2016. For this reason, the EU-LCI value has been used in the calculations.
- For ethylene glycol, the AgBB LCI value is set at 0.26 mg/m^3, whereas a newer EU-LCI value of 3.4 mg/m^3 was adopted in 2016. For this reason, the newest EU-LCI value is used in the calculations.

Table 3: Threshold limit values used in the calculations of limit values for selected substances

Substance	CAS no.	Used TLV (mg/m^3)	Comments	Reference used
Glycerine (glycerol)	56-81-5	10	Equal to the occupational exposure limit value	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	0.01761	Occupational threshold limit value (8-hour average, 40 hours, 45 years) based on 1/1,000 excess risk of lung function below normal	NIOSH, 2016
Acetyl propionyl (AP)	600-14-6	0.03808	Occupational threshold limit value (8-hour average, 40 hours, 45 years) based on 1/1000 excess risk of lung function below normal	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
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

Substance	CAS no.	Used TLV (mg/m^3)	Comments	Reference used
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

In Table 4 on the next pages, the proposal for limit values has been made for the 15 selected ingredients based on the calculation method and values presented in chapter 7. Please note that the calculations are made for a daily intake of 200 as well as 500 puffs, as described in chapter 7.

In Table 4 it is indicated with a light red background colour if the proposed limit values are exceeded by existing maximum levels of the substance identified in this review in e-liquids. Similarly, it is indicated with a light green background colour if the proposed limit values are *not* exceeded by existing maximum levels of the substance identified in e-liquids.

For 10 of the 15 substances where limit values in e-liquids are proposed, information about the actual concentration in e-liquids has been identified in this review. In 9 of 10 cases, the actual identified maximum concentration in e-liquids is higher than the proposed limit values, which indicates that there may be a need for limit values.

However, as the available information about the actual minimum concentrations measured in e-liquids reveals, it will be possible for some of the examined e-liquids to meet the proposed limit values for 8 of the 10 substances where measurements have been carried out.

Table 4: 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} = 5.33 m^3 (TLV 8h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 10 mg/m^3	16,000 (16 mg/mL or 1.6%)	40,000 (40 mg/mL or 4%)	Found in concentrations from 7 to 42% (solvent). Average content was 26% (Hutzler, 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 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 2.1 mg/m^3	10,080 (10 mg/mL or 1.0%)	25,200 (25 mg/mL or 2.5%)	Found in concentrations from 2 to 79% (solvent). Average content was 53% (Hutzler, 2014). A content of 79% equals 790,000 µg/mL assuming a density of 1.
Ethylene glycol	107-21-1	V_{air_daily} = 16 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 3.4 mg/m^3	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). Average content was 26% (Hutzler, 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} = 5.33 m^3 (TLV 8h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.01761 mg/m^3	28	70	Found in concentrations from 26 to 278 µg/day (Farsalinos et al., 2015), 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} = 5.33 m^3 (TLV 8h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.03808 mg/m^3	61	152	Found in concentrations from 20 to 432 µg/day (Farsalinos et al., 2015), 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 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.0005 mg/m^3	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 m^3 (TLV 24 h) $V_{Liquid\_per\_puff}$ = 150 puffs/mL n_{puffs} = 500/200 puffs Used TLV = 0.000005 mg/m^3	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. Flavourings are, however typically, used in low concentrations, i.e. below 0.1%.*
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* Prohibited in tobacco in Germany (Hutzler, 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%)	*No indication on concentration levels found in this review. Flavourings are, however, typically used in low concentrations, i.e. below 0.1%.*
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%)	*No indication on concentration levels found in this review. Flavourings are, however, typically used in low concentrations, i.e. below 0.1%.* 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 (Hutzler, 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%)	*No indication on concentration levels found in this review. Flavourings are, however, typically used in low concentrations, i.e. below 0.1%.* Regulated by European Cosmetics Directive
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, 2014). However, only identified in 12 out of 28 e-liquids examined.

9 Discussion and recommendations

In chapter 8 (Table 4) a preliminary proposal for a limit value for the concentration of 15 selected substances in e-liquids has been made. The limit values are given in µg/mL e-liquid. The limit values have been calculated based on existing threshold limit values for such as occupational exposure, outdoor concentrations for air, indoor emissions for building products etc. In many cases, the existing threshold limit values are based on long term systemic exposure, but may also be based on acute effects such as irritation. In general, the lowest existing threshold limit values were used for the calculation of proposed limit values for concentration in the e-liquids and in the calculations, a high number of puffs per day (500) has been used. It should, however, be emphasised that the number of 500 puffs per day is not considered as worst case – even though in the high end, as different chat forums for e-cigarette vapours indicate that even heavier vapours can be found. Furthermore, a limit value for 200 puffs per day has been calculated to compare the calculated limit values with the limit values for some substances set in the existing standards.

It should be stressed that the calculations performed in this report are made on a screening level, i.e. that a simple 'worst case' calculation has been made. This means that it is assumed 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.

The calculated proposed limit values for the 15 selected ingredients in e-liquids lie between 0.012 µg/mL e-liquid (for nickel) and 78,000 µg/mL e-liquid corresponding to 7.8 % (for menthol).

For 10 of the 15 substances where limit values in e-liquids are proposed, information about the actual concentration in e-liquids has been identified in this review. In 9 of 10 cases, the actual identified maximum concentration in e-liquids is higher than the proposed limit value. In some cases, the maximum concentrations found in e-liquids are a factor of 2-4 times higher than the proposed limit values in this report. In other cases, the maximum concentration found in e-liquids exceeds the proposed limit value by a factor of 25 to 200 – in one case even by a factor of 19,000 (for nickel). This fact suggests that limit values may be needed.

However, as the available information about the actual minimum concentrations measured in e-liquids reveals, it will be possible for some of the examined e-liquids to meet the proposed limit values for 8 of the 10 substances where measurements have been carried out. For example, benzaldehyde was only identified in 4 out of 28 e-liquids where its content was measured. This suggests that it will be possible for a large part of e-liquids on the market to meet the suggested limit value for benzaldehyde. Furthermore, the substances diacetyl and acetyl propionyl have been identified in e-liquids in concentrations below the proposed limit values. The extent of e-liquids below the proposed limit values is, however, unknown.
Some of the most often used ingredients and the ingredients used in the highest concentration (sometimes up to 79%) are the solvents glycerine, propylene glycol and ethylene glycol. The maximum measured concentrations of these solvents also exceed the proposed limit values (based on 500 puffs/day) by a factor of 26, 79 and 48 respectively for the listed solvents. Only for ethylene glycol, the minimum identified measured concentrations in e-liquids of 1% is below the proposed limit

value of 1.6%. However, according to the Afnor standard (2015b), ethylene glycol is restricted with 'no use'. This suggests that the use of the solvents in e-liquids needs further examination.
In contrast, the elements (lead and cadmium) are exceed by a factor of 2 to 3.4, i.e. the maximum identified concentrations (impurities) in e-liquids are just exceeding the proposed limit values. The maximum concentration of nickel identified in e-liquids is 226 µg/mL and exceeds the proposed limit value for nickel of 0.012 µg/mL by a factor of about 19,000. It must, however, be emphasised that for impurities such as lead, cadmium and nickel, it was only in about 3 to 15% of the measured cases (of 183 e-liquid measurements performed in Visser (2015)) that a content of these elements was identified above the level of quantification (LOQ), 5, 1 and 10 ng/mL respectively. Furthermore, it must be stressed that the proposed limit values for these three elements are all above the level of detection (used in Visser, 2015). This indicates that most of the e-liquids could comply with the proposed limit values for the elements lead, cadmium and nickel.

The British guidance document PAS (BSI, 2015) and the French Afnor standard (Afnor, 2015b) both contain some restrictions concerning ingredients in e-liquids – including some of the 15 selected ingredients for which limit values have been proposed in this report. The PAS document restricts the following ingredients in e-liquids:

- Diacetyl (DA) – shall not be used. No limit value is given.
- Acetyl propionyl (AP) – shall not be used. No limit value is given.
- Ethylene glycol – shall not be used, but might be present as contaminant in a maximum concentration of 0.1%, i.e. 1,000 µg/mL.
- Metals (including lead, cadmium and nickel) shall not be added, but may be present as impurities under toxicological supportable levels. No limit values are given.

The limit value proposed in this report for ethylene glycol is higher than the PAS limit value (about 16,000 µg/mL compared to 1,000 µg/mL in the PAS standard).

When comparing with Afnor limit values set for content in e-liquids (Afnor, 2015b), most of the limit values proposed in this report is lower than the Afnor limit values and in one case higher, but in the same order of magnitude. The limit values set in the Afnor (2015b) standard are:

- Diacetyl (DA) – shall not be used as an ingredient. Limit value 22 mg/L, i.e. 22 µg/mL.
- Ethylene glycol – shall not be used as an ingredient. No limit value set.
- Lead – limit value of 10 mg/L i.e. 10 µg/mL.
- Cadmium – limit value of 1 mg/L, i.e. 1 µg/mL.

The limit values proposed in this report for diacetyl are in line with the Afnor limit value, but higher (28 µg/mL compared to 22 µg/mL in the Afnor standard), for lead the proposed limit value is a factor of about 4 times lower, and for cadmium the proposed limit value is a factor of about 40 lower. The Afnor standard also restricts the use of ethylene glycol (no use); however, no limit value has been set. The limit value proposed in this report of about 16,000 µg/mL (i.e. 1.6%) suggests that the substance has no place in e-liquids as a solvent. Hutzler (2014) illustrated that ethylene glycol was used as a solvent replacing propylene glycol in some e-liquids.

The fact that many of the proposed limit values are exceeded by the maximum measured concentrations in e-liquids suggests that smoking e-cigarettes (heavy vapours of 500 puffs/day) for some e-liquids cannot be considered as safe, i.e. long term (or acute) health effects are likely to occur. However, the minimum measured

concentrations found in literature for some of the substances, also illustrate that the proposed limit values are not exceed for every e-liquid on the market, but that it is possible to meet the proposed limit values.

The fact that the proposed limit values for the most often used ingredients (the solvents glycerol and propylene glycol) are exceeded by a factor of 26 and 79 respectively (for the maximum measured concentrations), even suggest that smoking e-cigarettes in cases of more average vapouring behaviour cannot be considered as safe. Even the lowest measured concentrations found in literature for these two solvents are above the proposed limit values for the solvents.

As mentioned earlier, the proposed limit values are calculated by use of scenarios of high use of e-cigarettes as well as assumptions of a full intake of the substances contained in the e-liquids. This result is most likely an overestimation of the absorption of the substances in the e-liquids into the human body by smoking e-cigarettes and thereby calculation of lower limit values may be necessary. However, if no information is available concerning absorption of the substance in the body (no search for this has been made in the preparation of this report), a full absorption (100%) must be used as a precautionary principle.
Furthermore, it can also be discussed which limit values that should be used for the individual substances. In this report, the lowest limit values found have generally been used. The proposed preliminary limit values in this report should therefore be considered as a first approximation and the risk assessment presented needs further refinement in order to establish limit values for ingredients in e-liquids.

However, there is no doubt that smoking e-cigarettes is not risk-free, which has also been proven by a large study by Pissinger, who has carried out several reviews on the subject (Pissinger, 2014; Pissinger, 2015). In a letter to the Danish Parliament, Jørgensen and Pissinger (2016) argued against the sale of e-cigarettes in Denmark or at least a restriction of public smoking of e-cigarettes in line with ordinary cigarettes/tobacco. Actually, Jørgensen & Pissinger (2016) state that the use of e-cigarettes should never have been allowed as a new product on the market because of the content of carcinogenic, toxic and addictive substances. It may be a safer alternative compared to smoking of ordinary cigarettes/tobacco, but it is a huge problem if non-smokers take up the habit of vaping e-cigarettes, because they consider it to be safe. This is, however, also a political discussion and will not be addressed further in this report.

According to the Tobacco Directive, it is only allowed to use ingredients in e-cigarettes and e-liquids which do not pose a risk to human health in heated and unheated form. This review and reviews by Pissinger (Pissinger, 2014; Pissinger, 2015; Jørgensen & Pissinger, 2016) illustrate that ingredients are used in concentrations which cannot be considered as safe. This fact emphasises the need for limit values for several of the ingredients used in e-liquids.

Neither the GPS Directive nor the Tobacco Directive contains specific requirements for e-cigarettes and e-liquids. It is therefore important that the standards developed set the necessary chemical requirements (for all the necessary chemical substances contained in the e-liquids) as well as the correct limit values to protect the consumer from unwanted health effects from ingredients contained in e-liquids.

10 References

Afnor, 2015a. XP D90-300-1. Electronic cigarettes and e-liquids — Part 1: Requirements and test methods for electronic cigarettes. Afnor, March 2015. https://www.boutique.afnor.org/xml/1855861/false

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

Allen et al., 2015. Flavoring Chemicals in E-Cigarettes: Diacetyl, 2,3-Pentanedione, and Acetoin in a Sample of 51 Products, Including Fruit-, Candy-, and Cocktail-Flavored E-Cigarettes. Allen JG, Flanigan SS, LeBlanc M, Vallarino J, MacNaughton P, Stewart JH, Christiani DC. https://ehp.niehs.nih.gov/15-10185/

Api et al., 2015. RIFM fragrance ingredient safety assessment, Linalool, CAS registry number 78-70-6. Food and Chemical Toxicology (2015). doi: 10.1016/j.fct.2015.01.005 http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/78-70-6.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

BfR, 2015. Nikotinfreie E-Shishas bergern gesundheitliche Risiken. Nr. 010/2015, BfR, 23. April 2015 http://www.bfr.bund.de/cm/343/nikotinfreie-e-shishas-bergen-gesundheitliche-risiken.pdf

Bibra, 2014. EU classification of nicotine mixtures under CLP Regulation 1272/2008 (as amended and corrected). Bibra proposal, 30 June 2014. https://echa.europa.eu/documents/10162/4dedbc91-3a7f-4973-860b-faec6651db45

BMA, 2012. BMA calls for strong regulation of e-cigarettes. March 2012 (updated November 2014). British Medical Association.

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.

Burstyn, 2014. Peering through the mist: systematic review of what the chemistry of contaminants in electronic cigarettes tells us about health risks. http://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-14-18

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

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/

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, 2015. Biocides Human Health Exposure Methodology. October 2015. https://echa.europa.eu/da/about-us/who-we-are/biocidal-products-committee/working-groups/human-exposure

ECHA, 2016. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.15: Consumer exposure assessment Version 3.0 July 2016. https://echa.europa.eu/documents/10162/13632/information_requirements_r15_en.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 Regulation no. 1272, 2008. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1272-20170101&qid=1484732074435&from=EN

Farsalinos et al., 2015. 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

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.

JRC, 2013. European Collaborative Action. Urban Air, Indoor Environment and Human Exposure. Environment and Quality of Life. Report No 29. Harmonisation framework for health based evaluation of indoor emissions from construction products in the European Union using the EU-LCI concept. EUR 26168 EN.

Jørgensen & Pissinger, 2016. A letter from Danish Association for Public Health (Dansk Selskab for Folkesundhed) to the Danish Parliament dated January 15, 2016 as a reply to the Danish proposal on a Statutory order on placing e-cigarettes on the Danish market. *In Danish:* http://www.ft.dk/samling/20151/almdel/suu/bilag/328/1589713.pdf

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

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.

OECD SIDS, 2002. Glycerol, CAS no. 56-81-5. SIDS Initial Assessment Report For SIAM 14, Paris, France, 26-28 March 2002, UNEP Publications. http://webnet.oecd.org/hpv/ui/handler.axd?id=4b0a2d87-3183-40d4-84f5-0e118c647b19

OECD SIDS, 2003. Menthols, CAS No. 2216-51-5, 15356-60-2, 89-78-1, 1490-04-6. SIDS Initial Assessment Report, For SIAM 16, Paris, 27-30 May 2003. UNEP Publications. http://www.inchem.org/documents/sids/sids/MENTHOLS.pdf

Orr, 2014. Electronic cigarettes in the USA: a summary of available toxicology data and suggestions for the future. Orr MS. Tob Control, 2014; 23:ii18-ii22. http://tobaccocontrol.bmj.com/content/23/suppl_2/ii18.full

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

Pissinger, 2015. A systematic review of health effects of electronic cigarettes. Charlotta Pisinger, MD, PhD, MPH, Research Centre for Prevention and Health, Glostrup Hospital, Denmark. Document prepared for the World Health Organization, December 2015.

Poulsen & Merlin, 2017. Requirements for substances formed during the evaporation of e-liquids used in electronic cigarettes. FORCE Technology, Poulsen

PB and Merlin C. Ongoing project commissioned by The Consumer Council at the Austrian Standards Institute.

RIVM, 2014. General Fact Sheet. General default parameters for estimating consumer exposure - Updated version 2014. RIVM report 090013003/2014. J.D. te Biesebeek et al.. http://www.rivm.nl/dsresource?objectid=fd9e2199-e7ad-4641-8b97-aff66699c0c4&type=org&disposition=inline

RIVM, 2015. CLH report for nicotine. Proposal for Harmonised Classification and Labelling. Substance name: nicotine. RIVM, Bureau REACH, April 2015, version 2.0. https://echa.europa.eu/documents/10162/56df129c-42d3-4533-a828-e5d26a58bc63

Schober et al., 2014. Use of electronic cigarettes (e-cigarettes) impairs indoor air quality and increases FeNO levels of e-cigarette consumers. Shober W, Szendrei K, Matsen W, Osainder-Fuchs H, Heitman D, Schettgen T, Jörres RA, Fromme H. International Journal of Hygiene and Environmental Health 217 (2014) 628–637. https://www.ocf.berkeley.edu/~dshuster/e-Cigarettes/Schober_2014a.pdf

Schraufnagel et al., 2014. Electronic cigarettes. A Position Statement of the Forum of International Respiratory Societies. Scharufnagel DE, Blasi F, Drummond MB, Lam DCL, Latif E, Rosen MJ, Sansores R, Zyl-Smit RV on behalf of the Forum of International Respiroatory Societies. http://www.atsjournals.org/doi/abs/10.1164/rccm.201407-1198PP

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

Tierney et al., 2015. Flavour chemicals in electronic cigarette fluids. Tierney PA, Karpinski CD, Brown JE, Luo W, Pankow JF. Tob Control 2015;0:1-6. https://www.researchgate.net/publication/275053626_Flavour_chemicals_in_electronic_cigarette_fluids

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

US EPA, 2011. Exposure Factors Handbook 2011 Edition (Final). https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252

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.