



Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Final Opinion on
Additives used in tobacco products
(Opinion 1)
Tobacco Additives I



The SCENIHR adopted this Opinion by written procedure on 25 January 2016

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems, which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCENIHR

This Committee deals with questions related to emerging or newly identified health and environmental risks and on broad, complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies. Examples of potential areas of activity include potential risks associated with interaction of risk factors, synergic effects, cumulative effects, antimicrobial resistance, new technologies such as nanotechnologies, medical devices including those incorporating substances of animal and/or human origin, tissue engineering, blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields (from mobile phones, transmitters and electronically controlled home environments), and methodologies for assessing new risks. It may also be invited to address risks related to public health determinants and non-transmissible diseases.

Scientific Committee members

Michelle Epstein, Igor Emri, Philippe Hartemann, Peter Hoet, Norbert Leitgeb, Luis Martínez Martínez, Ana Proykova, Luigi Rizzo, Eduardo Rodriguez-Farré, Lesley Rushton, Konrad Rydzynski, Theodoros Samaras, Emanuela Testai, Theo Vermeire

Contact:

European Commission
Health & Food Safety
Directorate C: Public Health
Unit C2 – Health Information and Scientific Committees
Office: HTC 03/073L-2920 Luxembourg

SANTE-C2-SCENIHR@ec.europa.eu

© European Union, 2016

The Opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The Opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/policy/index_en.htm

ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this Opinion. The members of the Working Group are:

SCENIHR

Emanuela Testai (Chair and Rapporteur), Istituto Superiore di Sanità, Rome, Italy

Peter Hoet (Rapporteur), Katholieke Universiteit Leuven, Leuven, Belgium

Konrad Rydzynski, Nofer Institute of Occupational Medicine, Poland

Theo Vermeire, National Institute for Public Health and the Environment (RIVM), The Netherlands

External experts:

Urmila Nair, German Cancer Research Center (DKFZ), Germany

Reinskje Talhout, National Institute for Public Health and the Environment (RIVM), The Netherlands

All Declarations of Working Group members and supporting experts are available at the following webpage:

http://ec.europa.eu/health/scientific_committees/emerging/members_wg/index_en.htm

ABSTRACT

The main purpose of this scientific Opinion is to assist the Commission in identifying the additives that should be put on the priority list as foreseen by Article 6 of the Tobacco Products Directive 2014/40/EU (TPD).

The SCENIHR was asked to identify those additives, amongst the most commonly used additives by weight or number, that have one or more of the following attributes:

- a. Contributes to the toxicity or addictiveness of the products and/or increases the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
- b. Results in a characterising flavour;
- c. Facilitates inhalation or nicotine uptake;
- d. Leads to the formation of substances that have CMR (carcinogenic, mutagenic, repro-toxic) properties and/or increases the CMR properties in any of the products concerned (cigarettes/Roll-your-own) to a measurable or significant degree.

To compile the list of priority substances, the SCENIHR considered inter alia several lists of additives from the European Union Member States and used the list from the Netherlands (containing 1260 compounds) as a typical example. The selection from that list was carried out following these steps:

- 1) Additives were ranked according to the frequency of detection in different brands as well as the highest amount used in cigarettes, which were considered the first two criteria for selection; this reduced the number of chemicals to be evaluated to approximately 100 compounds.
- 2) An initial scan was carried out, considering the categories above (see also Article 6(2 a-d) in the TPD) and focussing on those present in tobacco and papers, and resulted in a preliminary selection of 56 additives for which a literature search for data on general characteristics of the compounds, toxicity data (including CMR properties), information about characterising flavour (potentially contributing to attractiveness), inhalation facilitation or increase in nicotine uptake (potentially contributing to addictiveness) as well as for data on pyrolysis products and their toxicity.

This method made it possible to identify a number of priority substances based on their hazard profile; therefore, a full risk assessment was not carried out.

After the selection of 56 additives based on the aforementioned criteria, the SCENIHR noticed that the list also includes compounds previously evaluated within the EU project "Public Information Tobacco Control" (PITOC), which were selected independently.

A data sheet was prepared for each chemical containing the most relevant, aforementioned information. At the end of the data sheet, a paragraph describes the criteria for inclusion in the priority list.

The information about the toxicological profile is often quite scant, and when available, data are generally limited to the oral route of exposure, especially for flavouring substances that are used by food industries or very rarely to the dermal route (when used in the cosmetic products). Data about inhalation toxicity are negligible, as well as data on kinetic behaviour, making inadequate any route-to-route extrapolation.

Another common feature for most of the additives is the scarcity of information on the exposure to additives, including exposures resulting from the combustion reactions' products. Data on pyrolysis of most of the individual additives are limited.

For most tobacco additives, direct information about their possible contribution to addictiveness and attractiveness does not exist, although information can be derived from the mode of action of the additive. Scant or no information was available on possible mixture toxicity; also due to lack of knowledge about all the components of the mixture and their levels, only a qualitative estimation of possible additive effects due to chemicals with the same effect could be made.

The list of priority substances consists of 30 entries corresponding to chemicals/groups of chemicals for a total of 48 single chemicals selected for the priority list. These selected compounds show one or more properties characterised in the 4 impact categories. To summarise:

- 17 substances were selected because they fall/are suspected to fall in the category: toxic in unburnt form, among which 6 are suspected of CMR potential.
- 14 substances were selected because they are suspected of facilitating inhalation or increasing nicotine uptake (mechanism possibly contributing to addictiveness to smoking).
- 19 substances were selected because they show a characterising flavour, one of the factors potentially contributing to attractiveness.
- 20 substances were selected because they are known or suspected of forming irritant, toxic and/or CMR chemicals after combustion.

It was concluded that the 6 substances, for which the CMR potential could not be ruled out would, be the first priority on the priority list, because according to the Tobacco Products Directive 2014/40/EU, Article 7 foresees the prohibition of using additives that have CMR properties in unburnt form.

Other possible criteria to further prioritise within the list of 30 priority substances/groups were considered, such as the possibility of contributing to more than one of the aforementioned categories and the possibility of forming CMR compounds after combustion.

Keywords: tobacco, addictiveness, additives, cigarettes, cigars, Roll-your-own, tobacco, smoking, toxicity, characterising flavour, facilitated inhalation, combustion products.

Opinion to be cited as:

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks),
Additives used in tobacco products, 25 January 2016.

TABLE OF CONTENTS

ABSTRACT	4
1 BACKGROUND	8
2 TERMS OF REFERENCE	10
3 SCIENTIFIC RATIONALE.....	12
3.1 Introduction	12
3.1.1 Possible effects induced by additives in tobacco product	12
3.1.2 Data gaps	14
3.2 Methodology	15
3.2.1 Information collection.....	15
3.2.2 Information evaluation	16
3.2.3 The compilation of the list of priority substances	17
3.3 Tobacco additives on the list of priority substances	21
3.3.1 Acetanisole	21
3.3.2 Aliphatic gamma-lactones	22
3.3.3 Ammonium compounds	27
3.3.4 Benzaldehyde.....	28
3.3.5 Benzoic acid and sodium benzoate	31
3.3.6 Benzyl alcohol	32
3.3.7 Caramel colours	34
3.3.8 Carob bean extract	35
3.3.9 Cellulose	36
3.3.10 Cocoa.....	37
3.3.11 Diacetyl	37
3.3.12 2-furfural	39
3.3.13 Geraniol	40
3.3.14 Glycerol	42
3.3.15 Guaiacol	43
3.3.16 Guar Gum.....	45
3.3.17 Linalool	46
3.3.18 Liquorice.....	48

3.3.19	Maltol	48
3.3.20	Menthol	50
3.3.21	Natural/ botanical extracts	52
3.3.22	Phenyl acetic acid	56
3.3.23	Piperonal	57
3.3.24	Propylene glycol.....	59
3.3.25	Sorbitol	60
3.3.26	Sugars	61
3.3.27	Titanium dioxide	62
3.3.28	Trimethyl (cyclohex-1-enyl)but-2-en-4-one (β -damascone)	63
3.3.29	Vanillin	65
3.3.30	Weak organic acids	65
3.4	Additional substances.....	77
3.4.1	Acetophenone	77
3.4.2	3,4-Dihydrocoumarin	78
3.4.3	Dimethoxybenzene	79
3.4.4	Ethylbutyrate	80
3.4.5	Ethyl maltol	81
3.4.6	4-hydroxy-2,5-dimethyl-3(2H)-furanone	83
3.4.7	Ionone (mixed alpha and beta isomers)	84
3.4.8	3-Methyl cyclopentane-1,2-dione	86
4	OPINION	87
5	MINORITY OPINION	109
6	CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS.....	110
7	ABBREVIATIONS AND GLOSSARY OF TERMS.....	112
8	REFERENCES.....	114
	Annex 1: Additives evaluated by the EU project 'Public Information Tobacco Control (PITOC).....	128
	Annex 2: Results of the literature search carried out by external company	129

1 BACKGROUND

The Tobacco Products Directive 2014/40/EU strengthens the rules regarding the reporting and composition of tobacco products. In addition to tightening the obligations of manufacturers to report on ingredients¹ contained in tobacco products. The Directive regulates permissible additives (or levels thereof) in order to improve the functioning of the internal market whilst guaranteeing a high level of public health.

A) Article 7 of Directive 2014/40/EU foresees in particular the prohibition of the following:

- 1) tobacco products with a characterising flavour. (Art 7(1))
- 2) tobacco products containing the following additives² (Art 7(6)):
 - a) vitamins or other additives that create the impression that a tobacco product has a health benefit or presents reduced health risks;
 - b) caffeine or taurine or other additives and stimulant compounds that are associated with energy and vitality;
 - c) additives with colouring properties for emissions;
 - d) for tobacco products for smoking, additives that facilitate inhalation or nicotine uptake; and
 - e) additives that have CMR³ properties in unburnt form.
- 3) tobacco products containing flavourings in any of their components such as filters, papers, packages, capsules or any technical features allowing modification of the smell or taste of the tobacco products concerned or their smoke intensity. Filters, papers and capsules shall not contain tobacco or nicotine. (Art 7(7))
- 4) tobacco products containing additives in quantities that increase the toxic or addictive effect, or the CMR properties of a tobacco product at the stage of consumption to a significant or measurable degree. (Art 7(9))

The provisions outlined above shall apply in the first stage to cigarettes and roll-your-own tobacco. The exemption for other product categories may be removed under certain conditions.

B) Moreover, in line with Article 6, the Commission shall develop and update a **priority list of at least 15 additives** contained in cigarettes and roll-your-own tobacco by May 2016. This list shall contain additives

- 1) for which initial indications, research, or regulation in other jurisdictions exist suggesting that they have one of the following properties:
 - a) contributes to the toxicity or addictiveness of the products concerned / increases the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
 - b) results in a characterising flavour⁴;

¹ 'ingredient' means tobacco, an additive, as well as any substance or element present in a finished tobacco product or related products, including paper, filter, ink, capsules and adhesives (TPD 2014/40/EU)

² 'additive' means a substance, other than tobacco, that is added to a tobacco product, a unit packet or to any outside packaging (TPD 2014/40/EU)

³ CMR - carcinogenic, mutagenic or toxic for reproduction

- c) facilitates inhalation or nicotine uptake; or
 - d) leads to the formation of substances that have CMR properties / increases the CMR properties in any of the products concerned to a significant or measurable degree; and
- 2) that are amongst the most commonly used additives by weight or number according to the reporting of ingredients.

For these priority additives, enhanced reporting obligations will apply in the form of comprehensive studies which shall examine for each additive whether it has any of the properties 1 a) to d) specified above. Those studies shall take into account the intended use of the products concerned and examine in particular the emissions resulting from the combustion process involving the additive concerned. The studies shall also examine the interaction of that additive with other ingredients contained in the products concerned. The results of these studies shall assist Member States and the Commission in their enforcement efforts regarding Art. 7.

The SCENIHR produced a scientific Opinion on the attractiveness and addictiveness of additives in 2010⁵. In light of the time that has passed since that Opinion and the need to address the current regulatory requirements, the SCENIHR is asked to address the questions outlined in the Terms of Reference below.

⁴ 'characterising flavour' means a clearly noticeable smell or taste other than one of tobacco, resulting from an additive or a combination of additives, including, but not limited to, fruit, spice, herbs, alcohol, candy, menthol or vanilla, which is noticeable before or during the consumption of the tobacco product (TPD 2014/40/EU)

⁵http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_031.pdf

2 TERMS OF REFERENCE

The main purpose of the requested scientific Opinion is to assist the Commission in identifying the additives that should be put on the priority list. The scientific Opinion can, however, also provide useful input for Member States and the Commission in their broader regulatory/enforcement activities (e.g. setting thresholds/banning of additive), in particular in areas where the knowledge base may currently still be limited. In particular, the Committee is asked the following:

Opinion 1:

Question 1. Based on scientific evidence (including a review of relevant scientific data) and other relevant information currently available (initial indications, regulation in other jurisdictions), the Committee is asked to identify - for each category separately - those additives that fall/are suspected to fall within the scope of the following categories:

- a. Contributing to the toxicity or addictiveness of the products concerned / increasing the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
- b. Resulting in a characterising flavour;
- c. Facilitating inhalation or nicotine uptake;
- d. Leading to the formation of substances that have CMR properties / increasing the CMR properties in any of the products concerned (cigarettes/roll-your-own) to a significant or measurable degree;⁶

The assessment should include for each of the additives identified a comprehensive description of the type of information supporting its identification as well as a description and quantification of the strength of the observed characteristic and the strength of the available evidence supporting this finding⁷. If the Committee identifies more than 20 additives for a category, the Committee is entitled to prioritise in the light of the criteria set out in this section. In this case, the description is limited to the top 20 additives per category, whilst the other additives can be listed without description.

The Committee is asked to also consider in its assessment the interaction with other ingredients contained in the products concerned and the emissions resulting from the combustion process involving the additive concerned as well as the intended use of the products. Relevant knowledge gaps should be identified.

As far as relevant information is available, the Scientific Committee is asked to identify within its assessment the most commonly used additives by weight or number. If additives belong to a single group of substances with identical or very similar properties, both the group of substances and the list of substances falling into that group shall be presented and the most relevant substance(s) within that group identified.

⁶ If an additive is included in Annex VI of Regulation (EC) No 1272/2008, its CMR-classification should be provided and considered as appropriate. Additives that have CMR properties in unburnt form should be identified/listed, but do not require a comprehensive description.

⁷ Registrations/assessments of relevant substances under Regulation (EC) No 1907/2006 should be provided and considered as appropriate.

When examining the composition of tobacco products and the use of individual substances, the Scientific Committee is invited to consult the data on additives reported by the tobacco industry under the Tobacco Products Directive 2001/37/EC, but may also consider additional data sources. Furthermore, the Committee is invited to consider during their assessment the lists of additives permitted/prohibited for use in tobacco products as implemented by certain Member States.

Question 2. Based on its assessment in point 1, the Committee is asked to establish a list of minimum 20 and maximum 30 additives that are suitable/recommended to be added to the priority list of additives in line with Article 6 of TPD 2014/40/EU. When establishing the list, the Committee shall consider the public health risks associated with the additives (actual or suspected), strength of the available evidence and to the extent possible, the frequency of use of the additives in tobacco products. The Committee should indicate as far as possible rankings of additives in light of the above and provide an explanation for its ranking⁸.

Opinion 2:

Question 3. Furthermore, the Committee is asked to advise the Commission on the type and criteria for comprehensive studies that should be requested from manufacturers to assess the relevance of the individual additives, considering inter alia the knowledge gaps identified in point 1 above and the interaction of the additive with other additives/ingredients. Advice is also sought on the most suitable methodologies to be used (including a structure of the reports that can be peer reviewed).

⁸Substances belonging to the same group of identical/very similar substances should be considered jointly.

3 SCIENTIFIC RATIONALE

3.1 Introduction

The smoking flavour of a tobacco product is due primarily to the types, grades and blends of tobacco employed. In addition, it was reported that the tobacco industry uses more than 500 different cigarette additives in the United States (USA), accounting for 10% by weight, which are claimed to improve taste (e.g. sugars, cocoa and liquorice) and reduce harshness of the smoke, (e.g. humectants such as glycerol and propylene glycol) (SCENIHR, 2010). Humectants keep the humidity of the tobacco product retaining water and avoiding the generation of an unpleasant harsh smoke typical of dry tobacco. Many other additives are used in small amounts, especially flavouring substances. Just as the blends and types of tobaccos used are determining factors in the design of a product, the flavourings greatly influence the quality and acceptability of the finished product.

In 2010, the SCENIHR adopted an Opinion aimed at examining criteria for classifying tobacco additives as addictive or attractive, and at evaluating their role for the creation or maintenance of dependence on tobacco products. The aim of the present Opinion is to assist the Commission in identifying the additives that should be put on the priority list in line with Article 6 of the Tobacco Products Directive 2014/40/EU by May 2016. This list shall contain additives amongst the most commonly used additives by weight or number contributing to the **toxicity** (including CMR properties), **resulting in a characterising flavour** (one of the factors potentially contributing to attractiveness of tobacco products), or leading to the **formation of toxic substances** including those having **CMR properties**. Some additives may fall into several of the above-mentioned categories.

The present Opinion uses the term additives in line with the definition in the Tobacco Products Directive 2014/40/EU (see Article 2(23)), i.e. 'additive' means a substance, other than tobacco, that is added to a tobacco product, a unit packet or to any outside packaging.

This Opinion will, therefore, not cover nicotine and its properties or any other natural tobacco components.

3.1.1 Possible effects induced by additives in tobacco product

Toxicity

Additives can be toxic in their unburnt form, with different target organs and mechanisms involved; interactions between additives and of additives with other constituents of tobacco can also occur, the tobacco product being a complex mixture, leading to the formation of other chemicals or increasing the toxicity of the mixture. In addition, and most importantly, **combustion of tobacco generates substances that may be toxic**. An example is provided by aldehydes, such as formaldehyde, acetaldehyde, propanal, 2-butenal, 2-methylpropenal, butanal, methylbutanal, furfural, benzaldehyde, methylfurfural, methoxybenzaldehyde (Adam *et al.*, 2006, Baker *et al.*, 2004), formed by the pyrolysis of various sugars and polysaccharides added to tobacco products. Sugars are natural components of tobacco (up to 20% in the tobacco leaf), but they are also added to tobacco products during manufacturing.

For example, if a tobacco or tobacco blend is low in sugar (e.g. in the case of air-cured tobaccos), the smoke will often be alkaline and give a harsh and irritating effect. Sugar is added to restore a chemical equilibrium between the acid-forming and base-forming constituents of the smoke. This balance of sugars, acids and alkaline constituents varies by types of tobacco and is carefully adjusted by the tobacco manufacturer to produce a mellow, full-bodied smoke. The heating of sugars in the tobacco product initiates caramelisation, generating secondary products that have an attractive smell and taste, but may be toxic.

Addiction and Attractiveness

For the concepts of addiction (or “dependence”) and attractiveness, this Opinion refers to the definitions given in the previous evaluation (SCENIHR, 2010). Addictiveness refers to the pharmacological potential of a substance to cause addiction, in line with the TPD definition as ‘the pharmacological potential of a substance to cause addiction, a state that affects an individual's ability to control his or her behaviour, typically by instilling a reward or a relief from withdrawal symptoms, or both’. In addition to the neurobiological characteristics of the substance itself, dependence potential is related to the dose, speed of absorption, metabolism, and the physical and chemical features of the formulation (WHO, 2007). Attractiveness refers to factors such as taste, smell and other sensory attributes of a product designed to stimulate use (WHO, 2007). Therefore, a potential contribution to attractiveness can be given by additives resulting in a characterising flavour, as defined in the TPD (‘characterising flavour means a clearly noticeable smell or taste other than one of tobacco, resulting from an additive or a combination of additives, including, but not limited to, fruit, spice, herbs, alcohol, candy, menthol and vanilla, which is noticeable before or during the consumption of the tobacco product’).

Among the many factors influencing attractiveness, including marketing actions intended to reduce concerns (e.g. with “light” branding), a very relevant one is the generation of product sensory characteristics using flavours, especially sweeteners.

Indeed, the flavours are added to the natural tobacco to deliver better taste, thereby possibly increasing the attractiveness of the products and providing a specific and standardised taste, which makes it unique and recognisable among the large variety of available brands. A unique product binds smokers. Because natural tobacco is subject to yearly variation of its taste, companies add chemicals (up to 40 or more substances per product) (SCENIHR, 2010) to compensate and mask this variation maintaining the specific taste of a certain product. The ‘flavour specialist’ has the task of improving, mellowing and modifying the tobacco aroma and taste to fit the desires of the consumers and preserve the unique taste of a specific product over time.

In addition to the use of flavours, the attractiveness of tobacco products may be increased in many different ways, generally inducing what is called a ‘pleasant experience of smoking’, e.g. decreasing the harshness of the smoke, or reducing lingering odour using flavours such as limonene to make smoking more acceptable to bystanders.

The addictive potency of tobacco products may be strengthened through diverse pathways:

- By increasing the bioavailability of nicotine, for example, by adding chemicals altering the pH of tobacco (e.g. alkalisating agents such as ammonium compounds). At pH >8, a

higher percentage of nicotine is in its free uncharged volatile form, which would, therefore, more easily pass the cell membrane (e.g. in the oral cavity and in the lung epithelium); however, a high pH also increases the nicotine/tar ratio (Wayne and Carpenter, 2009) as well as the harshness of smoke (Hurt and Robertson, 1998). Therefore, the alkalinisation should be balanced by adding weak acids. On the other hand, the high local buffering capacity of the lung-lining fluid causes free nicotine to be protonated in the deeper airways (Willems *et al.*, 2006), limiting the absorption.

- Increased nicotine bioavailability and addiction may also be associated with substances facilitating the inhalation of tobacco smoke, as in the case of additives with local anaesthetic effects such as menthol and thymol. Their action could decrease the perception of the smoke-irritating effects, which induces the smoker to inhale the smoke deeper and more frequently. A similar result might also be obtained using bronchodilators, such as theobromine (Bates *et al.*, 1999, Fowles, 2001), generated from cocoa, caffeine and glycyrrhizine (frequently used as tobacco additives). Additionally, the use of humectants, which reduce the harshness of the smoke, also increases the possibility of inhaling deeper and increasing the number of puffs (Wayne and Henningfield, 2008).

- Additives, which interfere with nicotine kinetics by, for example, increasing the absorption of nicotine, decreasing its biotransformation/elimination or indirectly potentiating the effect of nicotine on the nervous system, possibly increasing the addictiveness of tobacco products.

- The pyrolysis of sugar substances to acetaldehyde and more complex aldehydes may increase nicotine addictiveness, but the data are not yet conclusive, although an important role of inhibition of monoamine oxidases by tobacco smoke was repeatedly demonstrated (SCENIHR, 2010).

3.1.2 Data gaps

There are many data gaps on tobacco additives characteristics. One is a lack of information about toxicological profiles. When data are available, they are limited to the oral route of exposure, especially for flavouring substances that are used by the food industry, or more rarely to the dermal route, when used in cosmetic production. Many of the additives used in the manufacturing of cigarettes are approved for use in the US by the Food and Drug Administration: they are on the list of ingredients generally regarded as safe (GRAS) and/or are indicated as 'of no safety concern' by JECFA or EFSA when used at the actual levels of use in food; in many cases, they are also considered safe by FEMA (Flavour and Extracts Manufacturers Association). However, these evaluations apply to ingredients in foods or cosmetics that are ingested or topically applied. This exposure route differs significantly from the one typical for additives in tobacco, which are either transferred to inhaled smoke in pure form, or are combusted and converted via pyrolysis into potentially toxic products. Therefore, it is imperative to assess the possible risks of additives in tobacco by taking into account that inhalation is the relevant route of exposure.

Data on inhalation toxicity and/or kinetic behaviour although relevant (the latter for enabling route-to-route extrapolation) are limited. Inhalation exposure due to the large surface area in the lungs can have a profound effect on the addictiveness of a toxic product, as well as the inherent toxic potential of the additive.

The absence of an epithelial barrier similar to the gastrointestinal mucosa or to the skin usually corresponds to a higher percentage of absorption and consequently, a higher internal dose.

A common data gap is the knowledge about exposure to the additive(s). Indeed, exposure information should include the actual amount of the different additives in the tobacco product and should also take into account the combustion reactions' products. Data on pyrolysis during the actual condition of use are scant. Furthermore, no relevant information is available on potential mixture toxicity, because there is a lack of knowledge on all the components in the mixture. Some papers compare the toxicity of tobacco product with and without additives, showing little differences and claiming no mixture toxicity: however, the high background toxicity due to tobacco products without additives could possibly mask the additive effects.

Finally, for most tobacco additives, there is no direct information about possible effects on addictiveness, due to a lack of specific tests, but indirect information can be derived based on the mode of action of the single chemical used as additive.

3.2 Methodology

3.2.1 Information collection

To facilitate the task of the SCENIHR, the Commission contracted a search of published literature related to the compounds evaluated within the PITOC project, covering the period between 1 January 2012 and 31 January 2015.

A search of general issues of tobacco additives and toxicity, addictiveness and attractiveness was also conducted in the period starting from 2008 (i.e. after the adoption of the previous SCENIHR Opinion) ending 31 January 2015. The details for the search and the obtained results are included as Annex 2.

The results of the update of PITOC compounds were analysed by the SCENIHR members not previously involved in the PITOC Project.

Information on prioritised additives was collected on available open literature/websites and from evaluations previously carried out by other Committees/International Organisations (e.g. WHO, EPA, EFSA, JECFA) with a focus on the same topics reported in the factsheets:

A) Reported tobacco industry uses:

Function: Addressing why the additive is used in tobacco.

Concentration: If available, the amount (concentration) of the additive used and the frequency of use and in which products they were used was searched for.

B) Reported effects:

B.1 Toxicity

Addressing adverse effects induced by the additive and how it contributes to the toxicity of the product, especially indicating CMR (carcinogenic, mutagenic or repro-toxic)

properties. In addition, consideration was given to whether toxic or CMR pyrolysis products are formed, and if they are, what effects they have.

B.2 Potential contribution to Addictiveness

Addressing the properties of the additive (or its combustion products) to facilitate the inhalation of tobacco smoke (e.g. anaesthetic properties) or of increasing nicotine bioavailability (increasing uptake, decreasing clearance), potentially contributing to the addictiveness of the tobacco product.

B.3 Use potentially results in a characterising flavour

Addressing the properties of the additive (or its combustion products) to result in a characterising flavour, which is one of the factors influencing attractiveness of the tobacco products.

3.2.2 Information evaluation

For this Opinion on tobacco additives, a detailed toxicological evaluation was not conducted on each compound: the available information was collected and analysed to identify hazardous characteristics (belonging to the aforementioned features) in order to prioritise additives on a scientific basis. This does not mean that substances that are not on the list are safe: they may have just been excluded because they are used at a low level or there is scant information available about them.

For its Opinions, the SCENIHR generally uses a weight of evidence approach (SCENIHR, 2012) in its analysis of experimental, clinical and epidemiological evidence on effects on humans. Regarding this Opinion, in view of the need to build a priority list (mainly based on hazard identification) and not to conduct a hazard characterisation and risk assessment, weight of evidence was applied as follows:

- When weight of evidence on a specific endpoint (toxicity, characterising flavour, etc.) is strong or moderate indicating that a compound or its pyrolysis products can be of concern for human health when used as an additive in tobacco, the additive was considered as a good candidate to be included within the list.
- In instances where strong or moderate evidence only exists on a certain endpoint after oral or dermal exposure, it is still necessary to acquire more information on inhalation because this is the relevant route of exposure (often not studied). In addition tobacco use often includes pyrolysis that potentially results in the generation of potentially toxic compounds to be inhaled.
- When the weight of evidence is uncertain or when evidence is not suitable for weighing, no conclusion could be drawn. Since in this Opinion a low weight of evidence is an inclusion rather than an exclusion criterion, in those cases when there is some concern, more data should be produced to clarify the uncertainties.

Therefore, the substance has been selected for the priority list based on inclusion/exclusion criteria (as explained below), the available scientific evidence, and the expert judgement of the SCENIHR members.

3.2.3 The compilation of the list of priority substances

To compile the list of priority substances, the SCENIHR considered data reported by the industry in the context of ingredient reporting under Directive 2001/37/EC. Some of these lists of additives were also published by Member States such as Belgium⁹, the Czech Republic¹⁰, Germany¹¹ and the Netherlands¹². An additional list was received from the UK authorities when the work for drafting the Opinion was in an advanced status; some were taken from other jurisdictions (e.g. USA, Canada, and Brazil) as well as from data published by industry¹³. For practical reasons (not having available an EU-wide list) the comprehensive list from the Netherlands containing 1260 compounds was used as a typical example, as verified in light of data submitted by other Member States. Most of the additives selected in the priority list belong to those more frequently used in the other available sources considered.

The actual 'use and frequency' on an EU-wide basis (not available to the SCENIHR) is an issue to be considered by the Commission during preparation of the legislation.

No information about single specific additives other than the most used ones was obtained from the analysis of the papers retrieved from the literature search used for this Opinion. Therefore, the list from the Netherlands provided a representative basis for the selection which was carried out applying the following inclusion criteria: frequency of use, amount and toxicity (including CMR properties) of a specific additive in its unburnt form or via the formation of toxic substances after combustion; information on the possibility of resulting in a characterising flavour (one of the factors potentially contributing to attractiveness) and/or of facilitating inhalation and increasing nicotine uptake (possibly contributing to addictiveness).

Additives were ranked according to their average amount (expressed as % w/w) in tobacco products together with the frequency of use in different brands. To identify substances for the priority list, a first cut-off for frequency in a brand was used obtaining approximately 100 compounds.

An initial scan, which considered points in the article 6,2 a-b, focussing on those additives present in tobacco and papers and excluding those mainly used in non-combusted components (e.g. filters), resulted in a preliminary selection of 55 chemicals. A literature search was carried out for these selected compounds for 1) general characteristics, 2) toxicity data (including CMR properties) in the unburnt form, 3) information about properties resulting in a characterising flavour (one of the factors potentially contributing to attractiveness), facilitating inhalation or increasing nicotine uptake (potentially contributing to addictiveness of the tobacco products), and 4) data on pyrolysis products and their toxicity. Data were compiled for the identification of priority substances. Importantly, as already explained, this is not a thorough

⁹ <http://www.health.belgium.be/eportal/Myhealth/Tobacco/Fabrication/Database/index.htm>

¹⁰ <http://www.szpi.gov.cz/lstDoc.aspx?nid=11323>

¹¹ http://www.bmel.de/DE/Ernaehrung/Gesundheit/NichtRauchen/_Texte/Tabakzusatzstoffe.html

¹² <http://www.rivm.nl/Onderwerpen/T/Tabak>

¹³ <http://www.bat-ingredients.com/>

toxicological evaluation of the large number of selected compounds: the compilation of the priority list is mainly based on hazard identification.

Although the SCENIHR was aware of the work and the outcome of the EU project 'Public Information Tobacco Control' (PITOC), these compounds were not included on the priority list *a priori*. Instead, an independent selection was done based on the agreed inclusion/exclusion criteria, regardless of the PITOC results. After the selection it appeared that additives selected and evaluated by the PITOC were included in the list of the 55 chemicals selected by SCENIHR (Ammonium compounds, Carob bean extract, Cellulose, Cocoa, 2-furfural, Glycerol, Guar gum, Liquorice, Menthol, Propylene glycol, Prune juice concentrate, Sorbitol, Sugars, Vanillin), with the exception of Carob bean extract, which was *a posteriori* added to the 'first screening' list, therefore, including a total of 56 additives. Annex 1 includes the report prepared by the German Cancer Research Centre (DKFZ)¹⁴ and the Annex for the factsheets for professionals created by RIVM¹⁵ in the context of the PITOC project.

For the additives previously evaluated by the PITOC Project, only an update of the literature starting from 2012 was considered necessary, because the toxicological properties, addictiveness and flavour characteristics were already summarised in PITOC data sheets. Therefore, the reader is referred to the Annex for the factsheets for professionals that were created during that project. For those chemicals previously evaluated in the PITOC project, only the paragraph related to the rationale for inclusion is reported; for the data sheets, the link to the PITOC Project web site is provided. On the basis of the update of the literature for the period 2012-2014, it was concluded that – except for menthol and cocoa (for which additional text is provided to report the literature update) - no new relevant information was available that would require adaptation or changes of the report's conclusions. The evaluation of the papers retrieved for the update was carried out by the SCENIHR members not previously involved in the PITOC project.

The selection for the priority list was performed on the basis of unfavourable toxicological characteristics of the compounds in its unburnt form or of pyrolysis products, and/or based on possible available information about properties resulting in a characterising flavour (one of the factors potentially contributing to attractiveness), facilitating inhalation or increasing nicotine uptake (potentially contributing to addictiveness of the tobacco products). A data sheet was prepared for each chemical containing the most relevant information and including a paragraph describing the criteria for inclusion into the priority list.

Although most of these additives have GRAS and FEMA approval: notably, this approval does not apply to tobacco additives that are either transferred to inhaled smoke in pure form, or are burnt and converted into pyrolysis products, which could have a range of undesirable effects. Additives are added to cigarettes at well-defined concentrations and combinations, after intensive research by industry. However, most of this information is not available to the public.

¹⁴https://www.dkfz.de/de/tabakkontrolle/download/PITOC/PITOC_Additives_in_Tobacco_Products_Report.pdf

¹⁵<http://www.rivm.nl/dsresource?objectid=rivmp:185755&type=org&disposition=inline>

Some chemicals with very similar structures (i.e. aliphatic gamma-lactones) and/or properties (e.g. weak acids) were grouped together. For the aliphatic gamma-lactones possible criteria for prioritization within the group were identified on the basis of unfavourable properties, and possible representative additives within the group were identified. In most of the cases, this was not possible, because the information gathered was not sufficient to evaluate any priority among members of the group and they could be considered of equal concern: in this case the only criteria to be applied are the frequency and amount of use.

For chemically defined additives, it was possible to identify a unique CAS number, reported in the data sheet and in Table 1 in the Opinion; the SCENIHR is aware that other compounds with similar structure and different CAS number, potentially used as tobacco additives, could be relevant as well. However, since very different toxicological profile can characterise isomers of the same molecule, it is not possible to generalise. More details about possible grouping will be dealt with in Opinion 2.

Table 1: Examples of CAS numbers for natural compounds and mixtures

Additive or smoke component	CAS numbers
Cocoa	
Cocoa extract	84649-99-0
Cocoa powder	95009-22-6, 68916-17-6,
Cocoa extract	84649-99-3
Cocoa butter	8002-31-1, 68916-17-16, 95009-22-6
Cocoa Shells	8002-31-1
Cocoa distillate	94649-99-0
Liquorice	
Licorice root, extract, powder and fluid	68916-91-6, 8008-94-4 84775-66-6
Menthol	
l-Menthol	1490-04-6 2216-51-5
D-Menthol	15356-70-4
D/L-Menthol	89-78-1
Peppermint absolute	8006-90-4
Spearmint oil	8008-79-5
Vanillin	
Vanilla bean extract	121-33-5 8024-06-4
Vanilla oleoresin	8023-78-7
Vanilla fragrance	84650-63-5
Vanilla Tincture	8047-24-3

The identification with CAS number is an issue for natural compounds and mixtures for which some of the substances' names can be linked to multiple CAS numbers referring to different specific fractions and extracts corresponding to different CAS numbers.

Some examples are given in the following table. In general, papers published in the open literature do not state the specific CAS number of the tested additive(s) rendering it almost impossible to make a clear distinction. Considering the similar structures, the effects are expected to be rather the same, except for some fraction with different physicochemical properties possibly affecting bioavailability and specific endpoints.

For some of the initially selected chemicals, data were scant or, when available, they seemed not to indicate particular concern with respect to human health effects. Those chemicals were excluded from the priority list, although - being part of the initial selection - they are cited in the Opinion for transparency reasons as 'additional chemicals'.

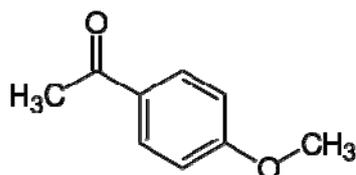
Finally, it must be emphasised that each tobacco additive (and combustion product) is only one component out of the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur, but cannot be adequately evaluated because of lack of available data.

3.3 Tobacco additives on the list of priority substances

3.3.1 Acetanisole

General

Acetanisole is an aromatic chemical compound with an aroma described as sweet, fruity, nutty and similar to vanilla. Acetanisole can sometimes smell like butter or caramel. It is used as a cigarette additive, fragrance, and flavouring in food. Acetanisole is found naturally in castoreum, the glandular secretion of the beaver.



CAS 100-06-1

Reported tobacco industry uses

Added as a flavour to tobacco (220 counts in NL ingredient lists, none in NTM (non-tobacco materials (NTM), total number of brands 4265), average (weight %) 0.0057 (0.013).

Health effects

JECFA Evaluation reported no safety concerns at current levels of intake when used as a flavouring agent in food GRAS status. CoE: ADI 1 mg/kg, agent in food http://www.inchem.org/documents/jecfa/jecval/jec_9.htm; Note that this is not sufficient proof of safety as a tobacco additive because the component is inhaled not ingested, and its combustion products may be toxic.

Toxicity

The oral LD₅₀ dose in mice is 820 mg/kg. At this dose, somnolence (general depressed activity), irritability, muscle weakness and weight loss are observed. According to the EC regulation criteria 1272/2008 (CLP), acetanisole is classified as Acute Tox. 4, H302 "Harmful if swallowed".

Acetanisole is a moderate skin irritant (>500 mg/kg) (Food and Cosmetics Toxicology, London, UK, FCTOD7, 1974), although it is not classified according to CLP criteria.

In 39-week intermittent inhalation studies of humans (1700µg/m³), increased pulse rate and blood pressure were observed (Makaruk and Vagonova, 1985). As tobacco products contain 0.0057%w/w, and a cigarette weighs approximately 700 mg, a cigarette contains approximately 0.04mg. If all the acetanisole is transferred to the cigarette smoke and inhaled, in 10 puffs of 55 ml smoke, there would be an estimated amount of 7000 µg per litre, which is higher than the dose given above. Even though this is a worst-case calculation, the fact remains that the margin of exposure is rather small.

In 13-week intermittent repeated-dose inhalation studies of rats (152 mg/m³/4H), changes in brain and coverings were reported, changes in serum composition (e.g. TP, bilirubin, cholesterol), and changes in enzyme inhibition, induction, or change in blood or tissue levels and other transferases (Makaruk and Vagonova, 1985).

From a pyrolysis experiment, it was concluded that intact acetanisole is likely transferred to the smoke (Purkis *et al.*, 2011).

Addictiveness

No report on addictiveness found.

Characterising flavour

Typically used as fragrance in amounts of 0.003-0.12 weight% (Monographs on Fragrance Raw Materials, 2013).

This is a similar range as added to tobacco products. Therefore, acetanisole may lead to a clearly noticeable flavour distinct from tobacco.

Rational for inclusion

Acetanisole is a known flavouring agent for food and is added to tobacco products for flavouring. More data are needed on the amount of acetanisole that imparts a noticeable flavour other than tobacco.

In order to make a toxicity risk evaluation, it is necessary to know the exposure level of acetanisole through cigarette smoking. Therefore, research is needed to determine the amount of acetanisole in mainstream cigarette smoke.

In human intermittent inhalation studies at concentrations relevant for tobacco smoke, increased pulse rate and blood pressure were observed. Because many toxicological inhalation data on acetanisole are missing, expected health effects after exposure to acetanisole from cigarette smoke remains unknown. Experiments, particularly on the effects on the cardiovascular system, respiratory tract, and CNS should be carried out.

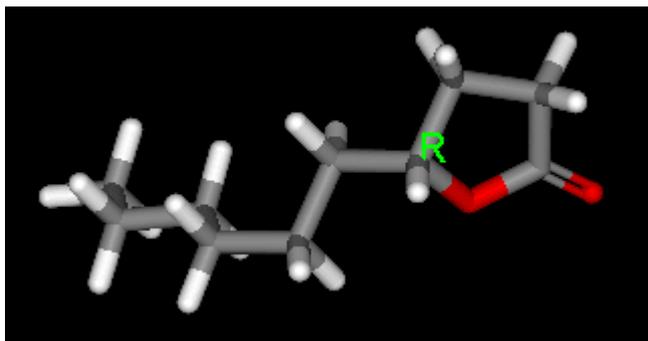
It is unclear if toxic combustion products of acetanisole are formed upon smoking a cigarette. From a pyrolysis experiment, it was concluded that intact acetanisole is likely transferred to the smoke (Purkis *et al.*, 2011).

3.3.2 Aliphatic gamma-lactones

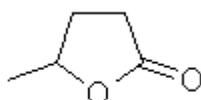
General

ALIPHATIC lactones: gamma-VALEROLACTONE, gamma-HEXALACTONE, gamma-HEPTALACTONE, gamma-OCTALACTONE, gamma-NONALACTONE, gamma-DECALACTONE; gamma-UNDECALACTONE, gamma-DODECALACTONE

Gamma-lactones are important flavour and aroma constituents in many natural products, frequently used as flavouring agents in many consumer products, including tobacco ones. Although both chiral enantiomeric forms occur in nature, the "R" chiral forms tend to be predominant (especially as the alkyl chain length increases). They were identified as tobacco/smoke components and additives in tobacco products.

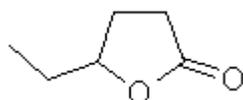


1) gamma-Valerolactone (C₅H₈O₂) CAS 108-29-2



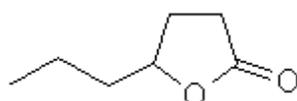
It is a weak-flavoured chemical found in products such as Virginia tobacco, cocoa, coffee, honey, peaches and wheat bread. The odour is often described as sweet, hay-like, coumarinic and coconut.

2) gamma-Hexalactone (C₆H₁₀O₂) CAS 695-06-7



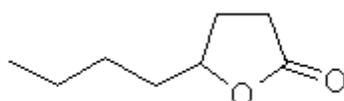
It occurs in products such as Burley tobacco, apricots, cocoa, grapes, grape brandy, mangos, peaches, raspberries, strawberries and wheat bread. The odour is described as "sweet, creamy, lactonic, tobacco and coumarin-like with green coconut nuances" and taste as "sweet, creamy, vanilla-like with powdery green lactonic nuances".

3) gamma-Heptalactone (C₇H₁₂O₂) CAS 105-21-5

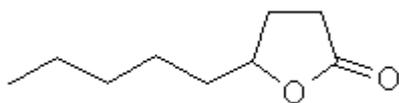


It occurs in products such as beer, liquorice, mangos, roasted filberts, papayas, peaches, strawberries, tea and wine. Odour is described as "sweet, coconut, coumarin, lactonic, creamy and powdery" and taste as "sweet, lactonic, creamy, coconut and coumarin, with nuances of milk and tobacco".

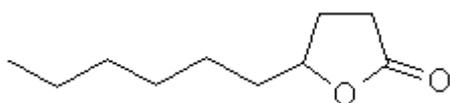
4) gamma-Octalactone (C₈H₁₄O₂) CAS 104-50-7



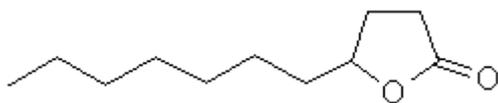
It occurs in products such as apricots, blue cheese, butter, cooked chicken, cooked pork, liquorice, milk, raspberries, roasted barley, roasted filberts (hazelnuts), roasted peanuts, roasted pecans, strawberries and tea. The odour is described as "sweet, creamy dairy with fatty and oily coconut nuances" and the flavour as "sweet, creamy, with coconut nuances".

5) gamma-Nonalactone (C₉H₁₆O₂) CAS 104-61-0

This material is often referred to as "Aldehyde C-18 (so-called)" by perfumers and flavourists, but it is a lactone, not an aldehyde. It occurs in products such as Virginia tobacco, asparagus, beer, cooked pork, liquorice, mushrooms, peaches, roasted barley, roasted filberts, tamarind, tea, wheat bread, whiskey and wine. The odour of this material is familiar to most people because it has been used as the sole "coconut" fragrance material in popular "Hawaiian" type sunscreen lotions. It is the basic characterising flavour component in artificial coconut flavours.

6) gamma-Decalactone(C₁₀H₁₈O₂) CAS 706-14-9

It occurs in products such as Oriental tobacco, apricots, blue cheese, butter, coconut milk (fresh), guava, mangos, peaches, plums, strawberries, tea and wine. The odour is described as "fatty, creamy, coconut, buttery, vanilla sweet, fruity, peach, with a sweet creamy character" and the taste as "fatty, oily, coconut, buttery sweet, fruity and peach-like".

7) gamma-Undecalactone (C₁₁H₂₀O₂) CAS 104-67-6

It occurs in products such as apples, apricots, butter, cooked pork, cooked rice, passion fruit, and peaches. The odour is described as "creamy, fatty, fruity, coconut, peach, lactonic, fruity" and the taste as "fatty, coconut, creamy, vanilla, nutty, and peach". This lactone has been the basis for artificial peach flavours for many years, before it was found in peach flavouring (in 1965) as a minor constituent.

8) gamma-Dodecalactone (C₁₂H₂₂O₂) CAS 2305-05-7

It occurs in products such as apricots, beer, blue cheese, cheddar cheese, cooked pork, milk products, peaches, pineapples, rum and strawberries. The odour is described as fatty, fruity and peach and the taste as milky-peach.

Reported tobacco industry uses

As taken from a patent for lactones as additives in tobacco (Tobacco product US 3996941 A), the use of lactones 'provides a product that will be acceptable to the consumer, particularly as regards flavour and aroma characteristics. A compound of the class described or mixtures thereof is added to tobacco or applied to a tobacco product

or its component parts in amounts from about 0.0005 to about 1.0 percent by weight of the tobacco or tobacco product. Preferably, the amount of additive is between about 0.001 and 0.1 percent by weight. However, while the additives are effective at low levels of concentration, the amount used will depend upon the amount of flavour and/or aroma desired and the particular compound or mixtures thereof used.'

The gamma-lactones' group is added as flavour to tobacco in the range between 41 and 407 counts in the NL ingredient lists, and between 0 and 84 in NTM, average (weight %) between 0.2528 and 0.0004.

As it can be derived from Table 2, the score according to the NL list regarding the count of Brand Name is: nona->octa->undeca->valero->epta>deca->dodeca; whereas in term of content (%w/w) hepta-lactone shows the highest value (0.2528 %), followed by nona- and octa- (0.017 %), being dodeca-lactone characterised by the lowest content and frequency.

Health effects

Some aliphatic lactones were evaluated by EFSA as flavouring substances used in food in the FGE.10 rev3. (EFSA 2012).

For simple saturated lactones, the ring-opening reaction and reverse cyclisation are in equilibrium, mainly controlled by pH conditions: in basic and neutral media, such as blood, the open chain hydroxycarboxylate anion is favoured while in acidic media, such as gastric juice and urine, the lactone ring is favoured. Both the aliphatic lactones and the ring-opened hydroxyl-carboxylic acids can be absorbed from the gastrointestinal tract: the simple low molecular weight uncharged lactones may cross the cell membrane more easily than the acidic form, which penetrates the cells as a weak electrolyte. The absorption is also high after administration via aerosol.

The hydroxycarboxylic acid obtained from lactone hydrolysis enters the fatty acid pathway and undergoes alpha- or beta-oxidation and cleavage to form acetyl CoA and a chain-shortened carboxylic acid. The carboxylic acid is then reduced by 2-carbon fragments until either acetyl CoA or propionyl CoA is produced. These fragments are then metabolised in the citric acid cycle. In addition, enzymes, such as lactonase and paraoxonase1, may catalyse the hydrolysis reaction of lactones.

Toxicity

According to the evaluation procedure applied to food flavourings, the conclusions are: aliphatic lactones are generally metabolised to innocuous products (many of which are endogenous in humans); at the estimated level of intake as flavouring substances (below the thresholds of concern of 1800 µg/person/day for structural class I), they are not expected to be genotoxic (EFSA, 2012).

Other data found in the literature support the overall negative results in genotoxicity testing, a very low acute oral toxicity (LD₅₀ generally higher than 5000 mg/kg bw in rats); some data on short-term repeated oral toxicity indicate a NOEL of 175-300 mg/kg bw per day; lower values indicated as >14; 50; 72 mg/kg bw per day (undeca-, hepta, and valero-lactone, respectively) corresponded to the highest dose tested in the study.

Gamma-decalactone was shown to have antimicrobial properties. This compound acts as a fluidising agent in living cells: it rapidly diffused into model phospholipid bilayers (within 2 min), modifying the general physical state and *in vivo*, the lactone strongly increased membrane fluidity in the model yeast *Yarrowia lipolytica* (Aguedo *et al.*, 2003). This property can have a possible influence in the fluidity of the lung epithelium.

Pyrolysis studies (Baker and Bishop, 2004; Purkis *et al.*, 2011) indications:

- gamma-Valerolactone 99 %-100 % of the pyrolysate contained gamma-valerolactone
- gamma-Hexalactone 99.6 % of the pyrolysate contained gamma-Hexalactone
- gamma-Heptalactone 99.2 % of the pyrolysate contained gamma- Heptalactone
- gamma-Octalactone 99.6 %-100 % of the pyrolysate contained gamma-Octalactone
- gamma-Nonalactone 99.7 %-100 % intact transfer rate of gamma-Nonalactone
- gamma-Decalactone 98.2 % of the pyrolysate contained gamma-Decalactone
- gamma-undecalactone 99.2 %-100 % intact transfer rate of gamma-undecalactone
- gamma-Dodecalactone 98.4 % of the pyrolysate contained gamma-Dodecalactone

In addition, gamma-valerolactone was reported to convert into aromatic hydrocarbons through catalytic pyrolysis. The catalysts and reaction conditions are both critical in maximising the hydrocarbon selectivity. Four zeolites, i.e. MCM-41, β -zeolite, ZSM-5 and HZSM-5 were tested in this work, among which HZSM-5 (Si/Al=25) was the most effective catalyst in both reactivity and selectivity. Under the reaction temperature of 500°C, the highest carbon yield of 56.71 % of aromatics was achieved from γ -valerolactone with HZSM-5 (Si/Al=25) as catalyst. Moreover, the HZSM-5 catalyst was recycled for five times without a significant decrease in product selectivity (Zhao *et al.*, 2012).

Addictiveness

The addictive effect of nicotine may be increased if the metabolism rate of nicotine is reduced. Reduction of the metabolic rate of nicotine, e.g. by inhibition of the metabolic enzymes involved in nicotine degradation, implicates a higher bioavailability of nicotine (nicotine is present in the body for a longer time or at a higher blood level). The additives gamma-heptalactone, gamma-valerolactone, gamma-decalactone, delta-decalactone, gamma-dodecalactone, delta-undecalactone and gamma-hexalactone are mild to weak inhibitors of CYP2A6, an enzyme within the P450 enzyme system, involved in the metabolism of nicotine (Juvonen *et al.*, 2000). However, with IC50-values in the range 560-12,000 μ M, it seems unlikely that these compounds will inhibit nicotine metabolism at the concentrations used in cigarettes.

Characterising flavour

Considering the low threshold odour detection reported for some lactones, it is possible that their use results in a characterising flavour.

- Gamma-valerolactone Odour Detection Threshold (in beer)= 10000 ppb
- Gamma-hexalactone Odour Detection Threshold (in water) = 1600 ppb
- Gamma-heptalactone Odour Detection Threshold (in water) = 400 ppb
- Gamma-octalactone Odour Detection Threshold (in water) = 7 ppb
- Gamma-nonalactone Odour Detection Threshold (in water) = 65 ppb
- Gamma-decalactone Odour Detection Threshold (in water) = 11 ppb
- Gamma-undecalactone Odour Detection Threshold = 950 ppb

- Gamma-dodecalactone Odour Detection Threshold (in water) = 7 ppb

Rational for inclusion

Regarding attractiveness, lactones are known flavouring agents for food and are added to tobacco products for flavouring (in addition to also being tobacco constituents) and providing coumarinic and coconut tastes.

More data are needed on the amount of lactones that imparts a noticeable flavour distinct from tobacco; notably, the threshold for odour detection in humans when dissolved in water is as low as 7-11 ppb for some gamma-lactones (octa-, dodeca- and deca-lactone, respectively).

Regarding addictiveness, some lactones including the ones listed here are mild to weak inhibitors of CYP2A6, one of the P450 isoforms involved in the metabolism of nicotine, although at high levels of exposure. In addition, gamma-decalactone strongly increases membrane fluidity of living cells.

To perform a toxicity risk evaluation, it is necessary to know the exposure level of lactones through cigarette smoking. Therefore, research is needed to determine the amount of lactones in mainstream cigarette smoke.

The toxicological profile of lactone *per se* is not of great concern, but gamma-valerolactone converts to aromatic hydrocarbons through catalytic pyrolysis. Although other studies indicate that lactones are mainly transferred as such in the smoke mainstream, and the aromatic hydrocarbons are formed in the presence of a catalyst not included in the tobacco product composition, the hazardous potential for lactones pyrolysis products cannot be totally dismissed. Additional pyrolysis experiments are recommended.

On the basis of the information regarding the characterising flavour, octa-, deca- and dodeca-lactones are likely to be the more relevant within the group. Decalactone and longer chain length lactones showed increasing amount of break-down products. On this basis it should be wise to select at least two representatives lactones within the group with short and long chain length. Another criterion for prioritisation among the group could be related to the frequency of use as well as the content, according to which gamma-hepta-lactone is used at the highest levels although less frequently than nona-, octa-, and undeca-lactone. However, the criteria related to frequency of use and content should be checked and adjusted by the Commission on the basis of an EU-wide database.

3.3.3 Ammonium compounds

Please refer to the PITOC Project factsheet

Rational for inclusion

In the Netherlands, ammonium compounds are rarely added. Ammonium Phosphate is used in the highest concentrations, with 13 counts in NL ingredient lists, 5 in NTM, total number of brands 4265, average (weight %) 0.068 (0.056). Nevertheless, ammonia is transferred to smoke from the ammonium compounds naturally present in tobacco.

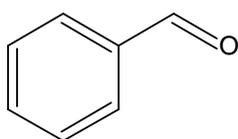
Regarding characterising flavour, ammonium compounds react with sugars during tobacco processing and smoking to form flavour compounds that have flavour-enhancing effects, such as deoxyfructosazine compounds, e.g. pyrazines, pyridines and pyrroles. Furthermore, DAP reacts with carbonyl compounds, such as formaldehyde and acetaldehyde in smoke, to reduce the harshness and irritation of cigarette smoking. More data are needed on the amount of ammonium compound that impart a noticeable flavour.

Regarding characterising flavour, there are some studies indicating that ammonium compounds increase the pH of the smoke which would consequently increase the amount of uncharged, or free, nicotine. Because the free base form is better absorbed, it has been hypothesised that it may result in faster and increased absorption of nicotine. However, results are inconclusive, and more research is needed to better understand the role that ammonium compounds play in nicotine transfer to tobacco smoke.

Regarding toxicity, ammonia is the major pyrolysis product generated from ammonium compounds during cigarette smoking. The critical effect of ammonia is irritation of the eyes, skin and upper respiratory tract. A risk assessment procedure using a Margin of Exposure (MOE) analysis concluded that a risk of effects on the respiratory tract epithelium due to ammonia could not be excluded. No thorough assessment on systemic effects was done. These conclusions were, however, based on ammonia levels in smoke that might also result from precursors in natural tobacco. More research in this area is needed.

3.3.4 Benzaldehyde

General



CAS: 100-52-7 (2-5), phenolic aldehyde

Benzaldehyde is an organic compound consisting of a benzene ring with a formyl substituent. It is the simplest aromatic aldehyde, with an almond-like odour, and can be extracted from a number of natural sources. Benzaldehyde is used chiefly as a precursor to other organic compounds, ranging from pharmaceuticals to plastic additives.

The presence of the aldehyde function makes benzaldehyde prone to oxidation yielding benzoic acid. Depending on the concentration, a disproportionation reaction can take place producing benzoic acid and benzyl alcohol. With amines, a condensation reaction can take place.

Reported tobacco industry uses

Benzaldehyde is added as flavour to tobacco (519 counts in NL ingredient lists, none in NTM, total number of brands 4265), average (weight %) 0.016 (0.13) .

Health effects

Benzaldehyde is used as a flavouring and fragrance in food, cosmetics, pharmaceuticals, and soap and is "generally regarded as safe". The JECFA reviewed benzaldehyde as a food additive and an acceptable daily intake of 0-5 mg/kg bw was established. They concluded that there would be no safety concerns at the current levels of intake when used as a flavouring agent. Note that this is not sufficient proof of safety as a tobacco additive, because tobacco additives are inhaled and not ingested and its combustion products may be toxic.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated a daily intake of 9300 µg/day in Europe and 36000 µg/day in the USA, which corresponds to 160 and 600 µg/kg bw per day.

Toxicity

Benzaldehyde was classified as a hazardous substance by the U.S. EPA, which evaluated the non-cancer oral data for benzaldehyde and derived a reference dose (RfD) of 0.1 mg/kg-day based on forestomach lesions, and kidney toxicity (hyperplasia and/or hyperkeratosis in the forestomach and degeneration or necrosis of the liver and tubular epithelium of the kidney).

The acute oral toxicity of benzaldehyde is low. Benzaldehyde is an irritant to skin, eyes and airways. Benzaldehyde may cause contact dermatitis (The Hazardous Substance Data Bank, 2004).

In patch tests using 5% benzaldehyde in Vaseline, positive reactions were observed in ten of 100 patients. Positive reactions occurred in patients with sensitivity to benzoic acid or vanillin (The Hazardous Substance Data Bank, 2004).

Benzaldehyde applied undiluted to intact or abraded rabbit skin for 24 hour under occlusion was moderately irritating.

After subacute inhalatory exposure, benzaldehyde causes CNS disturbances depending on the dose administered. Subchronic oral exposure to benzaldehyde (800 mg/kg/day or more) induces degeneration and necrosis of the cerebellum and hippocampus.

Benzaldehyde acts as a feeble local anaesthetic. It causes central nervous system (CNS) depression in small doses and convulsions in larger doses (Sax, 1984).

Epileptiform convulsions were observed in rabbits (Gosselin *et al.*, 1976).

Benzaldehyde has a sedative effect. Benzaldehyde (20-50 mg) decreases the motility in Swiss mice after a 1-hr inhalation period and after an induced over-agitation by i.p. application of caffeine (0.1%, 0.5 ml/animal) (Buchbauer *et al.*, 1993).

Benzaldehyde was administered by inhalation to Sprague-Dawley rats for 14 consecutive days (low level: 500 ppm; medium level: 750 ppm; high level: 1000 ppm). Throughout the experiment, significant hypothermia and a reduction of motor activity were observed in all rats exposed to benzaldehyde and were accompanied in high-level rats by a severe impairment of the central nervous system, as evidenced by abnormal gait, tremors, and a positive Straub sign (Laham *et al.*, 1991).

As $1 \text{ ppm} = 4.34 \text{ mg/m}^3 = 4.34 \text{ }\mu\text{g/l}$, for a smoker inhaling approximately 50ml/puff, and approximately 10 puffs, this would amount to $2.17 \text{ }\mu\text{g/cig}$. This is the same order as typical amounts present in mainstream cigarette smoke, which contains between 1-4.5 $\mu\text{g/cig}$ (Pang and Lewis, 2011). Therefore, the rats were exposed to much higher levels than those present in MSS.

Tobacco industry studies report that benzaldehyde, even at exaggerated inclusion levels in cigarette tobacco compared with commercial inclusion levels, showed no toxicological sequelae. Importantly, comparative toxicity studies are performed for cigarette smoke with and without the additive of interest (Coggins *et al.*, 2011).

Pyrolysis experiments with 291 single-substance ingredients, including benzaldehyde, were performed by the tobacco industry. Prior to these experiments, a set of pyrolysing conditions that approximates those occurring in the pyrolysis region of the burning cigarette was developed. The conditions include heating the sample at 30°C/sec from 300 to 900°C under a flow of 9 % oxygen in nitrogen. Pyrolysis of benzaldehyde resulted in a pyrolysate containing 94.9 % benzaldehyde, 0.2 % benzoic acid, 0.1 % ethyl benzoate and 4.8 % of 1 unidentified component (Baker *et al.*, 2004).

From a pyrolysis experiment it was concluded that benzaldehyde is likely transferred to the smoke at 200°C (74 % and 100 %): but at this temperature a significant amount (~26 %) oxidizes to benzoic acid (Stotesbury *et al.*, 1999). Both compounds appear resilient to further degradation (Stotesbury *et al.*, 1999). Intact recovery rates for benzaldehyde into the particle phase of mainstream smoke have been reported between 7 % and 9.4 % (Purkis *et al.*, 2011).

From a ^{14}C labelling study, it was concluded that the intact transfer (the proportion of additive in smoke that has not undergone any decomposition) of benzaldehyde is 100 % (Green *et al.*, 1989).

Addictiveness

There are no data available.

Characterising flavour

Typically used as fragrance in amounts of 0.001-0.08 weight % (Monographs on Fragrance Raw Materials, 2013). This is the same range as added to tobacco products. Therefore, benzaldehyde may lead to a noticeable flavour other than tobacco.

Rational for inclusion

Regarding characterising flavour, benzaldehyde is one of several other aldehydes present in cigarette tobacco and cigarette smoke. Benzaldehyde is a known flavouring agent for food and is added to tobacco products for flavouring. More data are needed on the amount of benzaldehyde that imparts a noticeable flavour.

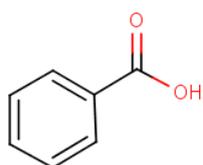
To perform a risk evaluation, it is necessary to know the exposure level of benzaldehyde through cigarette smoking. Therefore, research is needed to determine the amount of benzaldehyde in mainstream cigarette smoke.

Because only limited toxicological inhalation data on benzaldehyde are available, it is unclear what health effects to expect after exposure to benzaldehyde from cigarette smoke. Such experiments should be carried out, with a particular focus on the effects on the respiratory tract and CNS.

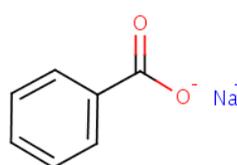
It is unclear if toxic combustion products of benzaldehyde are formed upon smoking a cigarette. Pyrolysis experiments performed by the tobacco industry indicate that benzaldehyde transfers largely intact into the smoke, with some formation of benzoic acid. Additional pyrolysis experiments are recommended.

3.3.5 Benzoic acid and sodium benzoate

General



CAS Registry Number 65-85-0



CAS Registry Number: 532-32-1

Benzoic acid is used as a pH adjustor and preservative in several products (food & cosmetics) (Nair B, 2001a). Benzoic acid and sodium benzoate were evaluated by SCCP as a preservative in cosmetics (SCCP, 2005).

Reported tobacco industry uses

Benzoic acid is an aromatic acid used in a wide variety of cosmetics as a pH adjuster and preservative. Sodium Benzoate is the sodium salt in benzoic acid and is used as a preservative and in a wide range of cosmetic product types (Nair, 2000).

Used up to 0.3 % (W/W) (SCENIHR 2010).

Health effects

Toxicity

Benzoic acid is a mild skin irritant, but sodium benzoate is not a skin irritant (SCCP, 2005). It is classified as an eye irritant.

Clinical data indicated that these ingredients can produce non-immunologic contact urticaria and non-immunologic immediate contact reactions, characterised by the appearance of wheals, erythema, and pruritus (irritant) (Nair, 2000; SCCP, 2005).

Benzoic acid and sodium benzoate rapidly metabolise and excrete via a common pathway within 24 hours. Systemic toxic effects on liver and kidney were observed. Benzoic acid and sodium benzoate have low acute oral and dermal toxicity with LD50

values >2000 mg/kg bw. The 4 hour-inhalation exposure of benzoic acid at 0.026mg/l/h also shows low acute inhalation toxicity.

The carcinogenicity studies were negative (Nair, 2000). It was shown that benzoic acid significantly increased the chromosomal aberration, sister chromatid exchange and micronucleus frequency (at 200 and 500 µg/mL) without changing the pH of the medium in a dose-dependent manner. This additive also significantly decreased the mitotic index (MI) at the highest concentration for 24 hours and 100, 200 and 500µg/mL for 48 hours (Yilmaz *et al.*, 2009). SCE negative in study (Jansson *et al.*, 1988). As summarised by SCCP (2005), various Committees concluded that the data reviewed for compounds in this group were sufficient to demonstrate lack of teratogenic, reproductive or carcinogenic potential.

Acceptable daily intakes were established by the World Health Organization at 5 mg/kg for Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. Benzoic Acid and Sodium Benzoate are generally recognised as safe in foods according to the U.S. Food and Drug Administration (WHO, 2002).

The available safety tests are not considered sufficient to support the safety of these ingredients in formulations where inhalation is a route of exposure. Inhalation toxicity data are needed to complete the safety assessment of these ingredients where inhalation can occur (Nair, 2001a).

Thermal decomposition gives rise to benzene, phenol, styrene (Winter and Barton, 1970).

Addictiveness

No data found.

Characterising flavour

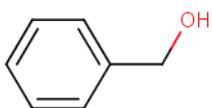
No data found.

Rational for inclusion

Benzoic acid and sodium benzoate should be considered as one group, using benzoic acid as the representative one. They pose no safety concerns *per se*, but it was reported that benzene, phenol and styrene can be formed following thermal decomposition. Because these are CMR compounds, data on pyrolysis related to benzoic acid as representative of the two are needed to carry out a proper evaluation.

3.3.6 Benzyl alcohol

General Information



CAS number: 100-51-6

Chemical class: aromatic organic alcohol

Physical/organoleptic properties: Colourless liquid with a slightly pungent, faint aromatic, fruity odour

Odour Threshold: 5.5 ppm

Local anaesthetic, designated fragrance allergen in the EU, vapours irritating to eyes, nose and throat, inhalation of vapours may cause drowsiness, dizziness and respiratory irritation (Pubchem and Inchem).

JECFA Evaluation (2001) reported no safety concerns at current levels of intake when used as a flavouring agent in food. FDA approved it as food additive; FEMA considered it as GRAS. Other uses: solvent, preservative, local anaesthetic, pharmaceutical aid, cosmetics and perfumery.

Reported tobacco industry uses

Maximum level of use 0.025 % w/w as flavour, solvent (RJRT, 2015).

Health effects

Toxicity

Potential effects of inhalation/vapour exposure: Nebulisers of bacteriostatic saline containing benzyl alcohol as a preservative can cause bronchitis in healthy adults. Inhalation of vapours may cause drowsiness, dizziness and respiratory irritation. Vapours irritating to eyes, nose and throat (Toxnet, 2015).

Benzyl alcohol is a local anaesthetic that could facilitate tobacco smoke inhalation.

Benzyl alcohol is on the list of fragrance allergens designated by the EU.

When heated to decomposition, it emits acrid smoke and fumes.

Industry publications reported influence on smoke chemistry, toxicity of several mixtures/ combinations of additives, which also included benzyl alcohol. However, no information is available on individual compounds.

The FEEDAP Panel concluded that the use of benzyl alcohol, benzoic acid and benzyl acetate is safe for all animal species at the maximum level of 125 mg/kg complete feed; Benzyl alcohol is considered harmful by inhalation. The FEEDAP Panel considered it prudent to treat all compounds under assessment as irritants to skin, eyes and respiratory tract, skin sensitisers and harmful if swallowed (EFSA, 2012).

Addictiveness

Benzyl alcohol is a local anaesthetic that can facilitate tobacco smoke inhalation. Possible additive anaesthetic effect, for example, with other compounds, e.g. menthol.

Characterising flavour

Odour slightly pungent, faint aromatic, fruity odour. Odour Threshold: 5.5 ppm.

Rational for inclusion

Benzyl alcohol is on the list of fragrance allergens designated by the EU. It is considered harmful by inhalation: vapours may cause drowsiness, dizziness, respiratory irritation and irritation to eyes, nose and throat. Moreover, it is a local anaesthetic and could facilitate tobacco smoke inhalation. When heated to decomposition, it emits acrid smoke and fumes.

3.3.7 Caramel colours

General

CAS number: 8028895

Chemical class: Caramel colours are produced by heating carbohydrates under controlled heat and chemical processing conditions. Caramel is a complex mixture of carbohydrate polymers and coloured aromatic compounds, which vary depending upon manufacturing process.

Physical/organoleptic properties: Dark brown to black liquids or solids with an odour of burnt sugar.

Odour Threshold: not available

General use: To impart a brown colour and associated characteristic caramel flavour to the foods to which they are added. Also used in cosmetics and pharmaceutical products.

Reported tobacco industry uses

Caramel, CAS number 8028895 FEMA 2235 Function: Flavouring, Maximum use 0.00003 % (BAT German domestic)

Caramel Colour, Max Level of Use in Any Cigarette Brand 0.01 % w/w, Function: Flavour (RJRT)

Health effects

Toxicity

Caramel colours are colouring substances authorised as food additives in the EU. The caramel colours are divided into four classes, Class I Plain caramel (E 150a), Class II Caustic sulphite caramel (E 150b), Class III Ammonia caramel (E 150c), and Class IV Sulphite ammonia caramel (E 150d), according to the reactants used in their manufacturing. The European Food Safety Authority has re-evaluated caramel colours toxicity and carcinogenicity in 2004, and established a group ADI of 300 mg/kg body weight (EFSA, 2004).

No information available on inhalation effect.

Industry publications have reported influence on smoke chemistry, toxicity of several mixtures/combinations of additives, which also included caramel. However, no information is available for the individual compound.

Caramel imparts flavour-influencing palatability. Combustion products would include aldehydes (can potentiate effect of nicotine) and toxic carcinogenic compounds.

Addictiveness

Combustion products would include aldehydes (aldehydes can potentiate effect of nicotine).

Characterising flavour

Odour of burnt sugar; Odour Threshold: not available

Rational for inclusion

The rational for inclusion is similar to the one related to natural extracts being a poorly characterised mixture of several to hundreds of chemicals; the composition is further dependent upon variable factors such as preparation methods. Although generally recognised as safe as food additives and flavours, this classification is not valid for their inhalation effects and pyrolysis products in tobacco smoke. The combustion/pyrolysis chemistry of caramel colours is still not well known in terms of their physiological, toxicological and synergistic additive effects to potentiate the harmful effects of tobacco smoke.

However, the pyrolysis of sugars (major component of caramel colours), was well reported. Upon combustion/pyrolysis at temperatures (up to 900°C) attained during smoking, these compounds, especially the carbohydrates, will give rise to a complex mixture of toxic, carcinogenic, mutagenic compounds, besides aroma/flavour compounds. Compounds formed include soothing agents (e.g. organic acids), flavours (e.g. caramel), facilitating nicotine delivery (e.g. aldehydes) and with CMR properties (e.g. PAHs, formaldehyde). The complex mixtures used as additives are a cause of concern and could contribute to CMR properties, addictiveness and characterising flavour of tobacco smoke.

Therefore, it is important to acquire more data on the exact composition of each of the undefined complex additives in unburnt and burnt forms.

3.3.8 Carob bean extract

Please refer to the PITOC Project factsheet

Rational for inclusion

Carob bean extract is rich in carbohydrates/sugars. It pyrolyses extensively and the combustion of the high carbohydrate/sugars leads to formation of carcinogenic and toxic compounds (e.g. benzene, polycyclic aromatic hydrocarbons, and phenol), aldehydes (acetaldehyde, formaldehyde, and acrolein), organic acids and caramel colour and flavours.

The aldehydes, acetaldehyde, acrolein and 2-furfural can be generated from the combustion of the sugars contained in carob bean extracts. Different combinations of aldehydes are generated and it is likely aldehydes other than acetaldehyde intervene directly or through the generation of new compounds in the smoke in the inhibition of MAO. Converging data indicate that MAO (monoamine oxidase) inhibitors contained in

tobacco and tobacco smoke act synergistically with nicotine to enhance addiction potential (SCENIHR 2010). In addition, toxic aldehydes are also formed. Carob bean extract has a sweet, fruity, chocolaty flavour and contributes to making smoking more attractive by improving flavour, thereby masking its bitter taste and reducing the harshness of smoking.

Carob bean extract is a chemically undefined complex additive containing hundreds of chemicals. Information on the exact chemical composition of this complex tobacco additive is lacking (e.g. carbohydrate, proteins/amino acids and fats, pH modifiers, and psychoactive chemicals). Moreover, analytical information on the number and concentration of flavour compounds including 'character impact compounds', present *per se* and generated upon heating is also not available in the public domain.

For example, pyrazines are important flavour impact compounds that are formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. Several pyrazines are also reported as additives to cigarettes to impart flavour in low tar cigarettes. (Alpert *et al.*, 2015).

This information can facilitate the assessment of the influence on the carob bean extract on palatability, pro-addictive effect and the interaction with other additives and tobacco chemicals.

3.3.9 Cellulose

Please refer to the PITOC Project factsheet

Rational for inclusion

Cellulose is used to prepare both the cigarette paper that wraps the tobacco and the filter (both the inner and outer layers). The cigarette paper is an important part of a cigarette. It controls how the tobacco burns and the amount of smoke. Generally, the more cellulose used, the greater the amount of smoke that is produced.

Reconstituted tobacco is made up of mashed tobacco stems and other parts of the tobacco leaf that would otherwise be discarded. Cellulose fibres are added to help bind and fill this reconstituted tobacco in cigarettes.

Cellulose does not transfer intact to the mainstream smoke, but undergoes extensive pyrolysis. Nearly 100 volatile products were reported from pyrolysis of cellulose. A complex mixture of toxic and carcinogenic compounds such as polycyclic aromatic hydrocarbons including benzo[a]pyrene, phenols, benzene, toluene, naphthalene, catechol, furan and furan derivatives, volatile aldehydes and levoglucosan, formaldehyde, acetaldehyde, acetone and acrolein were identified.

Formaldehyde, acetaldehyde, and acrolein are well-known upper respiratory tract and eye irritants. Aldehydes such as acetaldehyde, besides being toxic, are also reported to potentiate the effect of nicotine addiction. The generation of harman as a condensation product of acetaldehyde and biogenic amines may be responsible for the observed reinforcing effect of acetaldehyde.

In conclusion, the generation of carcinogenic and toxic compounds upon pyrolysis is well established. Further research to ascertain the composition of flavour compounds and

pro-addictive compounds (e.g. aldehydes) formed and their interaction with tobacco/smoke chemicals and their effect on MAO would be useful to ascertain the level of influence of this additive alone or in synergy on the addictiveness and palatability of the product.

3.3.10 Cocoa

Please refer to the PITOC Project factsheet

An interesting review was published on cocoa as a cigarette additive, which was in line with the PITOC evaluation and strongly supported its identification as a priority substance (Sokol *et al.*, 2014).

Rational for inclusion

Many forms of cocoa additives such as extracts and powders are used frequently and in relatively high amounts. Added as flavour or casing to tobacco (cocoa extract is the most abundantly used, with 847 counts in NL ingredient lists, none in NTM, total number of brands 4265), average (weight %) 0.105 (0.198). The maximum amount of cocoa as tobacco additive is around 1 % of the total tobacco weight (RIVM, 2012).

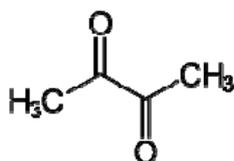
Regarding toxicity, the effects of cocoa inhalation through smoking have not been studied. The risk associated with the generation of combustion products produced upon cocoa pyrolysis has not been thoroughly studied and thus, conducting an adequate risk assessment for cocoa or its pyrolysis products is currently not possible.

Regarding addictiveness, several pharmacological effects of cocoa-derived ingredients were reported, including the bronchodilatory effect of theobromine and caffeine, which result in improved bioavailability of nicotine, although data available so far indicate that the content of theobromine per cigarette seems to be too low to have a bronchodilating effect on the lungs (SCENIHR, 2010). Furthermore, reaction products of tryptophan, phenylethylamine, tryptamine and tyramine, are thought to exert monoamine oxidase-inhibiting properties. In general, the pharmacologically active substances present in cocoa do not exclude a psychopharmacological effect in humans, owing to the low exposure concentrations and/or the inability of these substances to cross or reach the blood-brain barrier. Due to a lack of studies specifically on the psychoactive effects of cocoa added to tobacco, there is insufficient evidence that adding cocoa to tobacco makes cigarettes more addictive.

Regarding attractiveness, the addition of cocoa to tobacco is intended to enhance flavour. More data are needed on the amount of cocoa that imparts a noticeable flavour.

3.3.11 Diacetyl

General



CAS-nr:431-03-8

Other names: butanedione, butane-2,3-dione

Chemical structure: C₄H₆O₂

Chemical class: diketone

Diacetyl occurs naturally in alcoholic beverages and is added to some foods to impart its buttery flavour.

1 ppm = 3.58 mg/m³

Reported tobacco industry uses

Diacetyl occurs naturally in several types of tobacco and diacetyl is also added to cigarette tobacco to impart a buttery sweet taste and a buttery odour (Pierce *et al.*, 2014). Diacetyl was mentioned as a flavour compound that is generated during the heating of guar gum (German Cancer Research Centre, 2012) and sugars (Talhout *et al.*, 2006). In the NL list it is ranked at 53rd (amount: 0.0038 % w/w; frequency of use in the NL list: 296; Table 2)

Concentrations reported in cigarette mainstream smoke: 301–433 µg/cigarette (Fujioka and Shibamoto, 2006) and 250 to 361 ppm (Pierce *et al.*, 2014).

Health effects

Toxicity

The EC has declared diacetyl legal for use as a flavouring substance in all EU states. Currently, diacetyl is included on the U.S. Food and Drug Administration's (FDA) Generally Recognized as Safe (GRAS) list.

Exposure is widespread in food manufacturing industries in which workers handle diacetyl in the liquid form and are potentially exposed to diacetyl as vapours, fumes or adsorbed on particles, in the manufacturing process or at various stages of production. SCOEL (2014) concluded that airborne exposure to diacetyl in industries using butter-flavouring agents was associated with subclinical alterations of lung function and with fixed airway obstruction that may progress to a life-threatening bronchiolitis obliterans or bronchiolitis obliterans syndrome. SCOEL also concluded that diacetyl is able to cause subclinical to severe fixed airway obstruction, which is the critical health effect for recommending an OEL. Symptoms such as cough and shortness of breath are considered secondary to the obstructive lung disease. Even short-term peak flavouring exposures were reported to present a risk of lung damage. Average 8-hour diacetyl exposures as low as 0.02 ppm were measured in a work area where workers mixing butter flavourings with heated oil contracted bronchiolitis obliterans. In this case, peak exposures exceeded 80 ppm (Kreiss, 2007).

SCOEL accepted that there is uncertainty about the importance of the genotoxicity of diacetyl. There were no data on carcinogenicity.

By the SCOEL approach, a recommended 8-hour TWA-OEL of 0.02 ppm (0.07 mg/m³) was derived.

In order to prevent adverse health effects (mainly respiratory damage) which may arise due to exposure peaks not controlled by the above TWA limit, a STEL value of 0.10 ppm (0.36 mg/m³) was derived.

No data were found concerning transformation into toxic products after combustion.

Addictiveness

No data

Characterising flavour

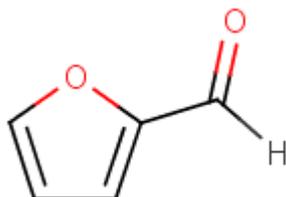
Diacetyl is added to cigarette tobacco to impart a buttery sweet taste and a buttery odour (Pierce *et al.*, 2014). It provides one of the characterising flavours of caramelised foods. The generation of such compounds can add to the olfactory cue and attractiveness of the smoking product and play a role in reinforcing nicotine dosing through helping ease of inhalation and possible olfactory cueing (DKFZ, 2012).

Rationale for inclusion

Diacetyl exposure may lead to serious lung disease after inhalation. For a proper risk assessment, it is necessary to better characterise the concentrations in mainstream smoke. SCOEL accepted that there is uncertainty about the importance of the genotoxicity of diacetyl. There were no data on carcinogenicity. In addition, it can create a characterising flavour, which can contribute to increasing attractiveness.

3.3.12 2-furfural

Please refer to the PITOC Project factsheet



Rational for inclusion

Furfural is used as a flavour and fragrance in the food industry as it has a sweet caramel-like flavour.

In tobacco, based on the NL list of ingredients, it is added as flavour to 71 tobacco products (none in NTM, total number of brands 4265). The average amount used is (weight %) 0.008 (0.030). Because 2-furfural has a distinctive flavour, it is possible that its use in tobacco results in a characterising flavour.

More data are needed on the amount of the compound that imparts a noticeable flavour.

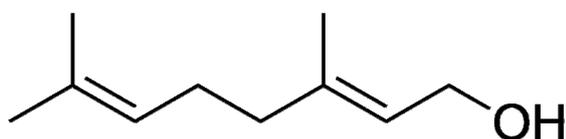
Regarding addictiveness, no data were reported to suggest that 2-furfural plays a role in smokers' addictiveness to cigarettes.

Regarding toxicity, IARC does not classify 2-furfural as carcinogenic to humans. Oral exposure to 2-furfural may lead to carcinogenicity in animal studies, but it has not been established if a similar effect occurs following inhalation exposure. Furthermore, 2-furfural showed a co-carcinogenic effect upon incubation with the potent mutagen benzo[a]pyrene, which is of concern because this component and several other mutagens are present in cigarette smoke. Further studies in this area are necessary. However, furfural *per se* does not pose any concern regarding genotoxicity (EFSA, 2011).

A risk assessment procedure using a Margin of Exposure (MOE) analysis concluded that risks of effects on the respiratory tract epithelium and of systemic inhalation toxicity from 2-furfural cannot be excluded. These conclusions were, however, based on 2-furfural levels in smoke that might also result from precursors in natural tobacco.

3.3.13 Geraniol

General



CAS number 106-24-1

Geraniol is an effective mosquito repellent flavour, although known to attract bees. It is part rose oil, palmarosa oil, and citronella oil, and occurs in small quantities in geranium, lemon, and many other essential oils. It is used as a flavouring agent by cosmetic industry (rose-like scent), as food flavouring (fruity taste) and as an additive in tobacco (and found naturally in well-aged tobacco).

The relevance of the impurities should be addressed: the formulations can contain the relevant impurity methyleugenol, which is a genotoxic carcinogen.

Reported tobacco industry uses

It is added as a flavouring agent to give a floral soapy aroma to tobacco products. The maximum percentage added to different brands: 0.1 % (SCENIHR, 2008).

It is added as flavour to tobacco 267 times in NL ingredient lists, 2 in NTM, average (weight %) 0.0015. The use of 23 ppm is reported by BAT.

Health effects

It was evaluated by EFSA as well as by SCCS in 2012 (EFSA, 2012 and SCCS, 2012). The information below is taken from these two documents.

Toxicity

Absorption, distribution and excretion of geraniol were likely rapid and extensive. Oral absorption was estimated to be probably higher than 80%. Accumulation is unlikely. The main metabolic pathways identified were oxidation reactions of the side chains to produce polar acidic metabolites.

Low acute toxicity was observed when geraniol acetate and geraniol extra were administered by the oral route to rats. No acute toxicity data are available on dermal and inhalation routes. Geraniol is considered to be a skin and eye irritant and has a high potential for skin sensitisation.

In bioactivation, hydroperoxides were not identified as metabolites, but other allergenic oxidation products (in particular, aldehydes and epoxides) were identified. Geraniol forms the aldehyde geranial, epoxy-geraniol, and also epoxy-geranial via autoxidation and metabolic oxidation, pathways of activation. Oxidation products of geraniol were identified as potent sensitisers in predictive animal tests. When bioactivation occurs, the risk of cross-reactivity should be considered. Cross-reactivity between geranial and geraniol, due to the metabolic oxidation of the alcohols to the aldehydes in the skin, was demonstrated.

Geraniol is included among the fragrance substances of clinical importance known to be a prehapten as well as a prohaptens. Sensitising compounds can be formed from geraniol by air oxidation (prehapten) and by metabolic transformation.

No levels that could be considered safe for the majority of consumers could be established from the available data.

The available dose elicitation studies indicate that a general level of exposure of up to 0.8 µg/cm² (0.01 %) may be tolerated by most consumers who have contact allergy to fragrance allergens. The SCCS considered that this level of exposure (0.01 %) could be efficient in limiting elicitation for any substance, unless there is substance-specific data, either experimental or clinical, proving the contrary.

In repeated oral toxicity studies with food grade geranyl acetate (29 % citronellyl acetate and 71% geranyl acetate) in mice and rats, mortalities and lipid vacuolation in the liver, kidney and heart were observed in mice and reduced body weight was observed in rats. The relevant short-term oral NOAEL is 1000 mg/kg bw per day (558 mg geraniol/kg bw per day; in 13-week rat and mouse studies). The relevant long-term NOAELs are 1000 mg/kg bw/day (558 mg geraniol/kg bw per day) for rats and lower than 500 mg/kg bw per day (279 mg geraniol/kg bw/day) for mice. No carcinogenicity potential was attributed to the mixture.

Geraniol did not induce gene mutations in *Salmonella typhimurium* and mammalian cells and equivocal response resulted in an *in vitro* clastogenicity test.

No data are available for reproductive and developmental toxicity with geraniol.

Geraniol is reported to be mainly transferred intact to smoke (85.6 %) with formation of about 30 minor compounds (Baker and Bishop, 2004) and 90.9 % in another study (Purkis et al., 2011).

Addictiveness

No information available.

Characterising flavour

Possibility that use results in a characterising flavour. Odour Detection Threshold (in water) = 40-75 ppb

Rational for inclusion

Geraniol is a known flavouring agent for food and is added to tobacco products for flavouring (one of the factors potentially contributing to attractiveness). More data are needed on the amount of geraniol that imparts a noticeable flavour other than tobacco. No data are available regarding addictiveness.

To perform a toxicity risk evaluation, it is necessary to know the exposure level of geraniol through cigarette smoking. Therefore, research is needed to determine the amount of geraniol in mainstream cigarette smoke. However, considering that the toxicological properties of geraniol are mainly linked to a high potential for skin sensitisation (in addition to skin and eye irritation), no levels considered safe for the majority of consumers could be established from the available data. Geraniol oxidation products (e.g. geranial, epoxy-geraniol, epoxy-geranial) are also potent sensitisers in animals. It could also be expected to be a respiratory sensitiser (although no information is available on this issue).

It is unknown if geraniol combustion products (about 10-15% of the additive) formed upon smoking a cigarette are toxic or not. Additional pyrolysis experiments are recommended.

3.3.14 Glycerol

Please refer to the PITOC Project factsheet

Rational for inclusion

Glycerol is added as humectant to tobacco (to help keep it moist). Its addition is mostly during the "casing" of the tobacco. The amount of glycerol present in cigarettes depends on the cigarette brand. The levels of glycerol added to tobacco in the EU is reported to be on average 1.1 % with a maximum level comprising 4.5 % of the total weight.

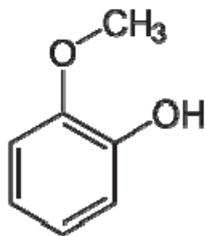
Regarding toxicity, it was reported by the tobacco industry that the transfer rate of glycerol to mainstream smoke is 12 %. A risk assessment procedure using a Margin of Exposure (MOE) analysis concluded that there are risks of effects on the respiratory tract epithelium from glycerol. No thorough assessment on systemic effects was done. Pyrolysis studies indicate almost 100 % intact transfer of glycerol (Baker & Bishop, 2004; Purkis *et al.*, 2011). However, it was found that less than 0.1 % of the blend glycerol is converted to acrolein in mainstream smoke for different cigarette designs and smoking regimes tested (Yip *et al.* 2010). Acrolein is a toxic compound that is highly reactive and causes irritation in the respiratory tract. The relationship between added glycerol and acrolein formation is unclear and further research is needed.

Regarding addictiveness, no data were reported to suggest that glycerol plays a role in smokers' addictiveness to cigarettes.

Regarding attractiveness, humectants are added to trap water, thereby keeping the moisture in the tobacco and preventing it from drying out. Glycerol is, therefore, considered to positively influence the attractiveness of cigarette smoking given that humidification improves the palatability of cigarettes. Glycerol does not have a strong flavour, and is, therefore, not expected to impart a noticeable flavour.

3.3.15 Guaiacol

General



CAS Number 90-05-1

Guaiacol (2-Methoxyphenol or 2-Hydroxyanisole) is a naturally occurring organic compound with the formula $C_6H_4(OH)(OCH_3)$. Although it is biosynthesised by a variety of organisms, this yellowish aromatic oil is usually derived from guaiacum or wood creosote. Samples can be colourless to amber crystals or an oily liquid, and darken upon exposure to air and light.

Guaiacol is present in wood smoke, resulting from the pyrolysis of lignin. The compound contributes to the flavour of many compounds, e.g. roasted coffee and tobacco products [FL-no: 04.005] with a smoky odour and caustic burning taste. It is also a precursor to various flavourants, such as eugenol and vanillin. Its derivatives are used medicinally as expectorants, antiseptics, and local anaesthetics (used in clinical dentistry as sedatives for the dental pulp, as disinfectants for caries, and as root canal medications). When heated to decomposition, bulk material emits acrid smoke and irritating fumes.

Reported tobacco industry uses

Added as flavour to tobacco 219 times in NL ingredient lists, 0 in NTM), average (weight %) 0.0015.

Maximum inclusion limit (%) in the UK: 0.15, 0.15, and 0.5 in cigarettes, cigars, and pipe tobacco, respectively.

Health effects

It was evaluated by JECFA (55th meeting). They concluded that there is no safety concern at current levels of intake when used as a flavouring agent in food. The same conclusions were confirmed by EFSA in 2008. Some guaiacol esters were also evaluated by EFSA in 2011.

According to REGULATION (EC) No 1272/2008, guaiacol is classified as:

H302 - Harmful if swallowed

H319 - Causes serious eye irritation

H315 - Causes skin irritation

Guaiacol is largely absorbed from the digestive tract and skin (after oral and dermal exposure) and stored in the blood, kidneys and respiratory organs. Guaiacol esters are easily hydrolysed; then guaiacol is metabolised by conjugation of the phenolic group

with sulphate or glucuronic acid at the hydroxy group followed by rapid excretion usually via urine.

The oral LD₅₀ of Guaiacol in rats is 520-725 (mg/kg bw).

When given subcutaneously, the fatal dose for guinea pigs and rats is 0.9 g/kg. Undiluted guaiacol was severely injurious to the eyes of rabbits (severe corneal necrosis and severe injury to conjunctival membranes). Guaiacol is a skin irritant (Pubchem) and it is also reported by the United States Pharmacopeia to be a respiratory tract irritant.

Subcutaneous administration (6.25-400 µL/40 g) of guaiacol to male Swiss Webster mice (30-45 g) produced tachycardia and hyperactivity, followed by sedation, hypnosis, high hypothermic effect (6.25-12.5 µL/40 g) and, in higher doses (25-400 µL/40 g), has a lethal effect. Necropsy showed hepatic and renal necrosis, pulmonary oedema, haemorrhages and bladder clotting (Martinez Enriquez *et al.*; 2009).

In line with its function as a dental pulp sedative, guaiacol, depending on the absorbed dose, can cause neurological, hemodynamic (shock), respiratory, metabolic (metabolic acidosis), renal (acute tubular necrosis), digestive and hematologic adverse effects (Martinez Enriquez *et al.*; 2009).

Other symptoms of exposure to guaiacol reported in humans include skin irritation and inflammation; dermatitis, nausea, vomiting, abdominal pain, headache, vertigo, dizziness, breathing difficulties, erythema, blistering and ulceration which can proceed to more severe damage with increasing doses.

A 2-hour exposure of mice to guaiacol vapours in a concentration ranging from 1.98 to 17.31 mg/L causes adverse effects and thus, indicates that the permissible concentration of guaiacol vapours is 0.02 mg/L (Ostrovskii, 1964).

Negative results were reported in the standard assay for reverse mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 incubated with guaiacol at up to 111 726 µg/plate (EFSA opinion on FGE 22, rev 1), although induction of SCE was reported in human lymphocytes at the highest concentration of the 5 tested (0.5 mmol/L corresponding to 62 µg/ml) (Jansson *et al.*, 1988).

Repeated exposure via the oral route of a structurally-related compound (4-methoxyphenol) was able to produce hyperplasia of the forestomach in rats (see EFSA FGE22.rev1), suggesting an effect at the site of contact, likely associated with the irritating properties of the compound on the gastrointestinal mucosa. A NOAEL of 0.25 % corresponding to 125 mg/kg bw/day was derived (Wada *et al.*, 1990). In one study, administration for 104 weeks caused an increase in atypical hyperplasia (male, 67 %, female, 37 %) followed by the presence of papillomas (50 %, 23 %) and squamous cell carcinomas (77 %, 20 %) (Asakawa *et al.*, 1994).

A series of 25 alkyl and alkenyl substituted guaiacols was identified in tobacco smoke condensate by GC-MS (Arnarp *et al.*, 1989), suggesting that guaiacol is likely transferred to the smoke as such. The pyrolysis of lignin (300-500°C) was indeed demonstrated to give rise to guaiacol (Kibet Jet *et al.*, 2012). Baker and Bishop (2004) reported that Guaiacol is transferred intact into smoke (92.5%) with some formation of guaiacol acetate, indanone, dimethoxybenzene and cinnoline.

Addictiveness

No report on addictiveness found, although its use as local anaesthetic can favour the smoke inhalation, thus potentially contributing to addictiveness.

Characterising flavour

There is a possibility that its use results in a characterising flavour: Odour detection threshold: 3-21ppb.

Rational for inclusion

Guaiacol is a known flavouring agent for food and is added to tobacco products for flavouring (one of the factors potentially contributing to attractiveness). More data are needed on the amount of guaiacol that imparts a noticeable flavour other than tobacco.

Its use as local anaesthetic can favour the smoke inhalation, thus potentially contributing to addictiveness.

To perform a toxicity risk evaluation, it is necessary to know the exposure level of guaiacol through cigarette smoking. Therefore, research is needed to determine the amount of guaiacol in mainstream cigarette smoke.

Guaiacol is a severe eye irritant, a skin irritant (Pubchem) and it is also reported to be a respiratory tract irritant. Other toxicological information on repeated exposure is scant. On the basis of results on structurally related compounds, effects are likely related to the irritation potential at the contact site, generating hyperplasia. Apart from the absence of mutagenicity tested with the Ames test, the only genotoxicity test on mammalian cells gave positive results (SCE in human lymphocytes). More data are needed for a better evaluation.

Pyrolysis experiments performed with lignin and finding many guaiacol derivatives besides guaiacol itself suggest that it transfers largely intact into the smoke.

3.3.16 Guar Gum

Please refer to the PITOC Project factsheet

Rational for inclusion

Guar gum is an extract of the seeds of the guar bean plant. Guar gum consists of high molecular weight polysaccharides and some amount of protein. Reconstituted tobacco is made up of mashed tobacco stems and other parts of the tobacco leaf that would otherwise be discarded. Guar gum (and its derivatives) is added to reconstituted tobacco in cigarettes. Guar gum is also used to prepare the cigarette paper that wraps the tobacco.

The amount of guar gum added to bind the tobacco can make up between 0.6-1.8 % of the total weight of the tobacco used in one cigarette. Guar gum is generally regarded as safe for use in food and cosmetics. However, guar gum does not transfer intact to the mainstream smoke, but undergoes pyrolysis, giving rise to toxic/carcinogenic (e.g. formaldehyde, benzo(a)pyrene and benzene) compounds.

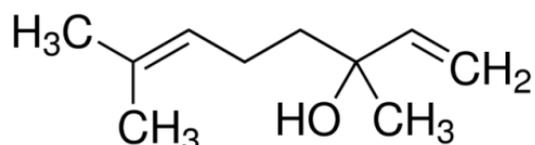
Irritating and toxic fumes, gases and acrid smoke can be formed when the additive is heated to decomposition.

Regarding flavours, it is well known that the thermal degradation of sugars and carbohydrates at lower temperatures as in foods contribute to complex aromas. Several flavour compounds were reported due to pyrolysis reactions of guar gum. These flavour compounds singly or in combination with the thousands of other smoke constituents can act synergistically and contribute to the attractiveness of smoking by improving smoke flavour, thereby masking its bitter taste, reducing the harshness of smoking, creating sensory cues, which all could contribute to the optimisation of nicotine dosing and enhance abuse potential.

Guar gum is hazardous when heated to decomposition, emitting acrid smoke and irritating fumes. Although some information on the effect of pyrolysis is available from the internal industry documents, further chemically defining this additive from the point of view as a tobacco additive and its pyrolysis products would help confirm/facilitate the assessment of the influence on the carob bean extract on toxicity/carcinogenicity, palatability, pro-addictive effect and the interaction/synergistic effect with other additives and tobacco chemicals.

3.3.17 Linalool

General Information



CAS number: 78-70-6 (This CAS number refers to a substance which contains between 10 and 80 % of each R and S isomer)

Synonyms: 3,7-DIMETHYLOCTA-1,6-DIEN-3-OL

Chemical class: Terpene alcohol (monoterpene)

Physical/organoleptic properties: colourless liquid with a pleasant, floral odour, lemon-like odour

General use: Flavouring agent, perfumery, cosmetics, chemical intermediate and pesticide

Local anaesthetic, designated fragrance allergen in EU, sedative, anxiolytic, analgesic, anticonvulsant and anti-inflammatory properties

Reported tobacco industry uses

Linalool is used as a flavouring, maximum use of 0.00001 % w/w (BAT)

Health effects

Toxicity

Linalool is a FDA approved food additive; FEMA GRAS, JECFA Evaluation deemed no safety concern at current levels of intake when used as a flavouring agent.

Linalool has sedative, anxiolytic, analgesic, anticonvulsant, anti-inflammatory, and local anaesthetic properties (Pubchem).

Linalool is on the list of fragrance allergens designated by the EU.

(-)-Linalool, the natural occurring enantiomer in essential oils, possesses anti-inflammatory, antihyperalgesic and antinociceptive effects in different animal models.

No information on combustion is available.

No information on inhalation toxicity is available.

Industry publications have reported influence on smoke chemistry and the toxicity of several mixtures/combinations of additives, which also included Linalool. However, no information is available for individual compounds.

Classified as a sensory additive and a functional groups-flavouring compound.

Linalool was also evaluated by the EFSA FEEDAP panel, which considered it safe for the environment based on their abundance in plant materials present in European countries. However, the FEEDAP Panel considered it prudent to treat all compounds under assessment as irritants to skin, eyes and respiratory tract, and as skin sensitisers (EFSA, 2012a).

Linalool is classified as Skin Sensitiser 1A; H317 Pure linalool is a weak sensitiser; however, it is vulnerable to autoxidation in air, which makes it a potent sensitiser. It forms stable hydroperoxides as primary oxidation products, which were shown to be the main allergenic agents. The autoxidation in air is an intrinsic property of linalool. Both human and animal data are available that demonstrate the skin sensitising properties of oxidised linalool. No information was reported regarding respiratory sensitisation (ECHA, 2014).

Addictiveness

No data available.

Characterising flavour

Odour: a pleasant, floral odour, lemon-like odour. Odour Threshold: 6 ppb

Interaction with other products

It can potentiate the effect of other analgesic additives, so possibly lower levels of such individual additives can be combined to give the same additive effect.

Rational for inclusion

Linalool has sedative, anxiolytic, analgesic, anticonvulsant, anti-inflammatory, and local anaesthetic properties. Linalool is on the list of fragrance allergens designated by the EU. It is classified as Skin Sensitiser 1A- H317.

No information on combustion available. No information on inhalation toxicity available, especially regarding sensitisation potential, which is a likely effect considering its properties.

Interaction with other additives: possible additive effect in combination with other additives that have pharmacological properties, e.g. menthol, which can increase the ease of inhalation or interact with other allergens.

3.3.18 Liquorice

Please refer to the PITOC Project factsheet

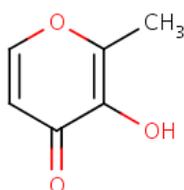
Rational for inclusion

Liquorice is a natural extract of the root of the liquorice (*Glycyrrhiza glabra*) plant – logically a not completely defined complex mixture of compounds. When heated to decomposition, it emits acrid smoke and irritating fumes. More than 400 compounds were isolated from *Glycyrrhiza* species. Liquorice extracts are used to improve the organoleptic properties of tobacco smoke, making the harsh cigarette smoke palatable, thereby enhancing the attractiveness of smoking. The taste and flavour of tobacco with added liquorice/liquorice root are described as sweet, woody and round. The major active principle of liquorice is the sweet tasting triterpene glycoside glycyrrhizin. Glycyrrhizin is a bronchodilator. It is not clear whether the levels present are sufficient for this effect, although a synergistic effect with other compounds in cigarette smoke maybe expected.

It is expected to pyrolyse extensively, but there is a lack of information on the pyrolysis products formed, which would help facilitate the assessment of the influence on toxicity/carcinogenicity. Additionally, the effect of liquorice on bronchodilation, alone or in combination with other additives and/or tobacco constituents needs to be ascertained to better understand its effect on the ease of inhalation of nicotine and other alkaloids, thereby potentiating addictiveness.

3.3.19 Maltol

General



CAS Registry Number: **118-71-8**

Structure: C₆H₆O₃

Synonym: 3-Hydroxy-2-methyl-4-pyrone, 118-71-8, 3-Hydroxy-2-methyl-4H-pyran-4-one, Palatone, Larixinic acid, Talmon.

Reported tobacco industry uses

- Smoking tobacco and smoking tobacco flavouring compositions containing hydroxy cyclohexenone derivatives (Light *et al.*, 1978).
- Tobacco flavouring with cyclohexadiene derivatives (Light *et al.*, 1979).
- Augmenting or enhancing the taste and/or aroma of consumable materials including tobaccos, perfumes and perfumed articles (Hall *et al.*, 1979).
- Flavouring substances include sugars, benzaldehyde, maltol, menthol and vanillin (RIVM, 2012).

Health effects

Toxicity

The inhibition of the response of GABAA receptors, which are the main inhibitory neurotransmitter receptors, by xanthin derivatives, allantoin, chlorogenic acid, 3-hydroxy-2-methyl-4-pyrone (maltol), trigonelline hydrochloride, and 2,3,5-trimethylpyrazine, may also contribute, in part, to CNS stimulation (Hossain *et al.*, 2003).

In cigarette condensates, different fractions were analysed: Maltol-induced SCE in human lymphocytes (as previously shown by (Stich *et al.*, 1981)). Maltol was previously reported as a mutagen, as determined by the Ames test (Bjeldanes and Chew, 1979), (Jansson *et al.*, 1986).

In its Opinion on FGE19 and FGE213 and FGE213 rev 1 (EFSA, 2008, 2009 and 2014), EFSA considered data for maltol [FL-no: 07.014] and concluded that the concern for genotoxicity could not be excluded. The conclusion was based on results obtained in a gene mutations test in bacteria and sister chromatid exchanges in human lymphocytes as well as *in vivo* induction of micronuclei in mouse bone marrow after maltol intraperitoneal application. Similarly, ethyl maltol induced gene mutations in bacteria.

More recently, maltol was tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 (Ballantyne, 2012): there was no evidence of any mutagenic effect induced by maltol in any of the strains, either in the absence or presence of S9-mix.

Maltol was also evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat liver S9-mix fraction as an *in vitro* metabolising system (Whitwell, 2012). The EFSA Panel concluded that maltol induced micronuclei *in vitro* in cultured human peripheral blood lymphocytes in the presence of rat liver metabolic activation (S9-mix) via a clastogenic mechanism of action (Whitwell, 2012). However, the Panel considered that the results observed in the absence of S9 were equivocal because the observed increases (which statistically significantly differed from the concurrent solvent control) were not reproduced in replicate cultures.

Maltol was evaluated in an *in vivo* micronucleus assay and liver Comet assay in male Han Wistar (HsdHan:WIST) rats, 6 rats per dose group (Beevers, 2013). Considering that maltol was shown to induce micronuclei in mouse bone marrow after intraperitoneal injection, the Panel concluded that the available negative findings could not rule out the concern for genotoxicity for maltol (EFSA, 2014). Indeed, data provided to prove systemic availability in the negative studies were considered inconclusive due to data inconsistency. According to EFSA, because the micronuclei induced by maltol in mice were analysed after intraperitoneal application, a micronucleus assay after oral application should be required in addition to an *in vivo* Comet assay to clarify the genotoxic potential of maltol (EFSA, 2014).

Addictiveness

No data available.

Characterising flavour

Considering the patents described above, maltol seems to be an important factor in the flavouring of tobacco, because maltol enhances the taste.

Reported as flavour, concentration: 220 ppm (Baker *et al.*, 2004); 0.005 % W/W (Thomsen *et al.*, 2010).

Rational for inclusion

Following the EFSA report on maltol (FGE19 and FGE213 and FGE213 rev 1 - EFSA, 2008, 2009 and 2014), the concern for genotoxicity could not be excluded. Therefore, maltol will be on the priority list until data on its genotoxicity is clarified. In addition, possible effects on the CNS must be clarified.

3.3.20 Menthol

Please refer to the PITOC Project factsheet

There are published reports on menthol after the PITOC evaluation. The DKFZ position paper from 2013 supported the proposal for a revision of the EU Tobacco Product Directive, which includes a ban on menthol. Support for this is that 1) menthol increases the attractiveness of tobacco products, 2) menthol targets youth and 3) menthol may promote smoking initiation (DKFZ, 2013). This is included in the section on menthol in the PITOC-report. Additional findings are presented below.

Rational for inclusion

Menthol is one of the most commonly used tobacco additives worldwide. It is a monocyclic terpene alcohol that is used primarily for its chemosensory effects of creating perceptions of a cooling minty taste and smell. Menthol is added at a continuum of concentrations, from imperceptible amounts to levels imparting different levels of a characterising flavour.

In addition, several additives and formulations are used to simulate menthol effects. Menthol induces anaesthetic and sensory effects, facilitates deeper inhalation and adds to the impact of nicotine.

Menthol is a multifunctional additive. It is an effective anaesthetic, antitussive agent that may increase the sensation of airflow and inhibit respiratory rate, thereby allowing increased lung exposure to nicotine, tar and toxic constituents, while masking reactions like coughing or other early warning signs of respiratory disease. It may increase the absorption and lung permeability of smoke constituents, thereby increasing nicotine and carcinogen uptake. It may also decrease nicotine/cotinine metabolism leading to higher doses of nicotine. It is one of the additives that was originally added to create the impression that a tobacco product has health benefits and/or reduced health risks. It affects multiple sensations including taste, aroma and tactile smoothness, and enhances abuse liability. Its pharmacological actions reduce the harshness of smoke and the irritation from nicotine, and may increase the likelihood of nicotine addiction in adolescents and young adults who experiment with smoking and make it more difficult to quit.

In 2011, the FDA Tobacco Products Scientific Advisory Committee (TPSAC, 2011) concluded that menthol 1) impacts youth initiation, 2) contributes to adults continuing to smoke, and 3) has an adverse impact on public health by increasing the numbers of smokers with resulting premature death and avoidable morbidity. Finally, they concluded that the "removal of menthol cigarettes from the marketplace would benefit public health in the United States" (TPSAC, 2011; FDA, 2011).

Independently, the US Food and Drug Administration undertook a thorough review and concluded that the data suggested that menthol use is likely associated with increased smoking initiation by youth and young adults, greater addiction, greater signs of nicotine dependence and less likelihood of successfully quitting smoking. These findings, combined with the evidence indicating that menthol's cooling and anaesthetic properties may reduce the harshness of cigarette smoke and the evidence indicating that menthol cigarettes are marketed as a smoother alternative to non-menthol cigarettes, make it likely that menthol cigarettes pose a public health risk above that seen with cigarettes without menthol (FDA, 2013). The review concluded that although there is little evidence that menthol cigarettes *per se* are more toxic than menthol-free cigarettes, adequate data indicate that menthol presence is associated with increased smoking initiation and greater addiction, especially among young people, as confirmed later by the studies of Nonnemaker *et al.* (2013) and Brennan *et al.* (2015). Indeed, smokers usually using menthol cigarettes develop greater nicotine dependence, which is likely associated to the anaesthetic properties that reduce the harshness of smoke. In addition, menthol cigarette smokers are less successful quitting smoking (Smith *et al.*, 2014). Recent perception studies confirm earlier work showing that smokers, especially young adults, perceive menthol cigarettes as less harmful (Brennan *et al.*, 2015; Wackowski and Delnevo, 2015).

With regard to toxicity, Noriyasu *et al.* (2013) exposed cell cultures to menthol and non-menthol smoke and found that cell death was significantly enhanced by mentholated smoke, whereas menthol alone was inert. This suggests a synergistic effect with other smoke-compounds and requires further study.

A recent study conducted in mice showed that menthol at low concentration strongly suppressed respiratory irritation due to acrolein and cyclohexane, which are smoke irritants in naïve mice. Additionally, menthol suppressed irritation by tobacco smoke in mice. Menthol increased blood cotinine levels, which is a biomarker of nicotine uptake. Thus, menthol appears to suppress smoke-induced irritation, making it easier to inhale

smoke and increasing the dosage of nicotine. Due to the similarities in menthol's pharmacology in humans, experiments in animal models suggest that beginning smokers likely prefer menthol-containing cigarettes because their respiratory tract is less irritated. At the same time, these smokers are exposed to higher levels of nicotine and become addicted faster and are less likely to quit smoking (Ha *et al.*, 2015).

At lower application levels, menthol can be used to increase smoothness and reduce harshness in cigarette smoke. This is likely the main reason for use of menthol as an additive, also in "non" menthol brands. Therefore, research to ascertain the physiological and pharmacological impact of low menthol and its interaction with other chemicals, interaction with nicotine, on palatability and inhalation of smoke/nicotine, etc. is recommended.

3.3.21 Natural/ botanical extracts

It is worth mentioning that (natural) extracts are most often composed of a complex mixture of different (often unknown) chemical compounds. Together, these compounds often make up the overall characteristic flavour and taste of the extract. Considering the toxicological/physiological effects, the mixtures and not the individual compounds are evaluated.

3.3.21.1 Fenugreek

General

Synonyms: fenugreek (*trigonella foenum graecum* L.) extract, resin, & absolute

CAS number: 84625-40-1

Chemical class: N/A

Physical properties: Complex mixture, dark brown paste.

General use: Flavouring agent. Fenugreek, CASRN: 68990-15-8

Trigonella foenum-graecum seeds contain mucilage, trigonelline, 4-hydroxyisoleucine, sotolon, diosgenin, phenolic acids, and protodioscin.

Reported tobacco industry uses

Fenugreek extract, maximum use 0.00451 % w/w as flavouring (BAT-Germany)

Fenugreek oleoresin, maximum use 0.00068 % w/w as flavouring (BAT-Germany)

Health effects

Toxicity

Fenugreek is "generally recognized as safe" (GRAS) as a flavouring by the US Food and Drug Administration.

However, this classification is not valid for inhalation effects and pyrolysis products when used as a tobacco additive. When used as a medicinal product, it is generally well tolerated in adults, but gastrointestinal side effects such as diarrhoea and flatulence may

occur. Allergic reactions, exacerbation of asthma, and a 14 % decrease in serum potassium have been reported (Toxnet).

Neurotoxicity: The alcoholic (TA), aqueous (TQ), petroleum ether (PE), alkaloidal (TK) and glycosidal (TG) extracts of fenugreek; fenugreek oil (FO); diosgenin (DI) and trigonelline (TR) were evaluated for their neuropharmacological activities in albino Wistar rats. All the extracts and active principles except TQ showed significant CNS stimulant activities, while TQ alone showed a significant CNS depressant activity. The results indicated that the active compounds present in fenugreek seed extracts had significant CNS stimulant and depressant activities (Toxnet).

It is a skin irritant. When heated to decomposition, it emits acrid smoke and irritating fumes (Guidechem).

Industry publications reported data on smoke chemistry and the toxicity of several mixtures/combinations of additives, which also included Fenugreek extract, oil, oleoresin and tincture.

No information available on inhalation effects.

No information available on combustion products.

Addictiveness

No information available.

Characterising flavour

Odour/Flavour: adds body, nutty, maple, sweet

Odour Threshold: N/A

3.3.21.2 Fig extract

General Information

CAS number: 90028-74-3

CoE number: 198

Chemical class: Complex mixture (*figus carica* l. extract), high in natural monosaccharides glucose and fructose. Reducing sugars are approximately 85% to 90% of dry solids

Physical properties: colour - amber to dark brown.

General use: Fig Concentrate used as a natural colouring and anti-staling agent, as well as a flavour enhancer. Fig concentrate is also widely used by the tobacco industry to provide a special flavour-aroma character and retain freshness in the finished tobacco product.

Reported tobacco industry uses

- Fig Juice concentrate used at <0.0001 % as flavour (RJRT);

- Fig juice concentrate: maximum level of use 0.00220 % w/w as flavouring (BAT).

Health effects

Toxicity

Industry publications reported data on smoke chemistry, toxicity of several mixtures/combinations of additives, which also included fig extract/juice concentrate.

Combustion products from the high sugar/carbohydrate concentration would include aldehydes (can potentiate effect of nicotine), flavour compounds (increase palatability) and toxic carcinogenic compounds (detailed in PITOC report, e.g. for plum extract).

Addictiveness

No information available.

Characterising flavour

Flavour: A fruity characterising fig-like flavour.

3.3.21.3 Prune juice extract

Please refer to the PITOC Project factsheet

Rational for inclusion

Chemically, prune juice is a complex mixture of hundreds of chemicals, 95 % carbohydrates largely sugars, soluble gums, amino acids (impart flavour), ammonia organic acids (impart smoothness), etc. It is used as a flavour/casing ingredient. The combustion of the inherent sugars can lead to the formation of carcinogenic polyaromatic hydrocarbons, a variety of aldehydes, such as acetaldehyde, acrolein, 2-furfural and a mixture of organic acids.

Prune juice concentrate is reportedly used alone or in combination with other natural flavours or commercial tobacco flavour improvers to "smooth or mildly sweeten the smoke and to blend the various natural smoke flavour ingredients".

Data gaps: This additive upon pyrolysis gives rise to carcinogenic and toxic compounds. Additional studies to ascertain the interaction of the many inherent chemicals as well as the pyrolysis products in increasing palatability and potentiating of nicotine compounds would facilitate the assessment of prune juice concentration palatability, pro-addictive effect and the interaction with other additives and tobacco chemicals.

3.3.21.4 Rum

General Information

CAS number: 91450-09-8 (From JTI). Others are also reported in publications.

Chemical class: distilled alcoholic beverage.

Physical properties: Complex mixture, high percentage alcohol and many flavour compounds.

Odour Threshold: N/A

General use: Used also as flavouring in foods and alcoholic drinks.

Reported tobacco industry uses

Rum or rum extract are used as flavour at the level QNE 0.01% by JTI Germany. Not on the list for Germany (BAT, PMI, RJRT).

Health effects

Toxicity

No relevant information on the impact of rum as a tobacco additive on inhalation.

No relevant information on the impact of rum as a tobacco additive on combustion.

Industry publications reported data on smoke chemistry, toxicity of several mixtures/combinations of additives, which also included rum.

Rum imparts flavour, will increase palatability.

Addictiveness

No information available.

Characterising flavour

Odour: rum-flavour, many flavour compounds and odour threshold N/A

Rationale for inclusion of natural extracts

Natural/botanical concentrates/extracts/resins (e.g. from several fruits - fig, plum, raisins, fenugreek, carob, cocoa, caramel, rum, etc.) form a large number of tobacco additives. They are poorly characterised complexes of several to hundreds of chemicals; the composition further depends upon variable factors influencing botanical source and preparation methods. Although generally recognised as safe as food additives and flavours, this classification is not valid for their inhalation effects and pyrolysis products in tobacco smoke. The combustion/pyrolysis chemistry of each of these additives is not well known in terms of their physiological, toxicological and synergistic additive effects to potentiate the harmful effects of tobacco smoke.

However, many of the botanical extracts have a rich carbohydrate/sugar content together with varying amounts of proteins, amino acids and other flavour compounds. The pyrolysis of this class of compounds has been well reported. Upon combustion/pyrolysis at temperatures (up to 900°C) attained during smoking, these compounds, especially the carbohydrates, give rise to a complex mixture of toxic, carcinogenic and mutagenic compounds, as well as aroma/flavour compounds. Compounds formed include smoothing agents (e.g. organic acids), flavours (e.g. caramel), facilitating nicotine delivery (e.g. aldehydes) and with CMR properties (e.g. PAHs, formaldehyde). Moreover, pyrazines are important flavour impact compounds that are formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. Several pyrazines are also added as additives to cigarettes

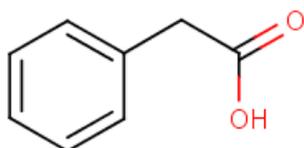
to impart flavour to low tar cigarette (Alpert *et al.*, 2015). For more details, see plum extract, fenugreek, sugars and cellulose.

The complex mixtures used as additives cause tremendous harm and contribute to CMR properties, addictiveness and attractiveness of tobacco smoke. Therefore, it is important to collect more data on the exact composition of every undefined complex additive in unburnt and burnt forms.

It was not possible to prioritise among the natural extracts, because the information available for each of them is scant, and hampered by their unknown and complex composition.

3.3.22 Phenyl acetic acid

General



Synonyms: Benzyl carboxylic acid, alpha-Toluic acid.

CAS number: 103-82-2.

Chemical class: aromatic organic acid.

Physical/organoleptic properties: Glistening white crystalline solid with leafy crystals; sweet honey-like.

Acute hazard symptoms: exposure can cause redness of skin and redness and pain in the eyes, inhalation can cause cough/sore throat; decomposes upon burning and produces irritating fumes.

Reported tobacco industry uses

Phenyl acetic acid used as flavouring at 0.00038 % w/w (BAT German domestic).

Phenyl acetic acid maximum use level 0.0001 % w/w in tobacco as flavouring (PMI German).

Health effects

Toxicity

FDA approved food additive; FEMA GRAS, JECFA Evaluation (2001): No safety concern at current levels of intake when used as a flavouring agent.

Acute hazard symptoms: Inhalation - cough/sore throat; Skin - redness; Eyes - redness and pain. It decomposes upon burning, producing irritating fumes. No information is available either on inhalation effect or on combustion products.

Industry publications reported data on smoke chemistry, toxicity of several mixtures/combinations of additives, which also included phenylacetic acid. However, no information is available for individual compounds.

It was evaluated by the EFSA FEEDAP Panel, which considered it prudent to treat it as an irritant to skin, eyes and respiratory tract and as a skin sensitiser (EFSA 2012b).

REACH dossier classifies phenyl acetic acid as an eye irritant.

Only 51.6 % of the parent compound transferred intact, with benzaldehyde and ethyl-phenyl acetate accounting for a further 44.5 % of the pyrolysate. The remaining 4 % of the pyrolysate was made up of 12 compounds (Baker and Bishop, 2004).

Addictiveness

No data available

Characterising flavour

Odour: Floral odour

Odour Threshold: 10000 ppb

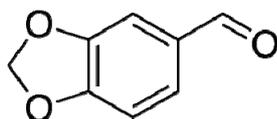
Rational for inclusion

Phenyl acetic acid is considered as a potential respiratory irritant by EFSA, thus possibly causing damage to the respiratory tract following inhalation. In addition, it decomposes upon burning, producing irritating fumes. More data are needed on those two issues, in order to exclude the respiratory irritation potential.

No/scant information is available on inhalation toxicity and combustion products.

3.3.23 Piperonal

General



CAS 120-57-0, phenolic aldehyde.

Piperonal, also known as heliotropin, is an organic compound that is commonly found in fragrances and flavours. The molecule is structurally related to other aromatic aldehydes such as benzaldehyde and vanillin. Piperonal naturally occurs in various plants, such as dill, vanilla, violet flowers, and black pepper. Piperonal has a floral odour similar to that of vanillin or cherry. It is commonly used to add vanilla or almond nuances, generally imparting balsamic, powdery, and floral aspects.

The presence of the aldehyde group means that piperonal can undergo reductive, oxidative and nucleophilic addition reactions; the dimethylenedioxy group makes it sensitive to hydrolysis. The aromatic core contributes to the lipophilic character of the molecule.

Reported tobacco industry uses

Added as flavour to tobacco (591 times in NL ingredient lists, none in NTM, total number of brands 4265), average (weight %) 0.031 (0.096).

Health effects

A FAO/WHO Expert Committee on Food Additives evaluated piperonal as a food additive. The conclusion based on the current levels of intake was: no safety concern. Note that this is not sufficient proof of safety as tobacco additive, because tobacco additives are inhaled and not ingested, and its combustion products may be toxic.

The JECFA estimated the oral daily intake of piperonal in Europe to be 1700 µg/day and in the USA to be 3200 µg/day (Joint FAO/WHO Expert Committee on Food, 1967).

Toxicity

The acute oral toxicity of piperonal is low (NOEL in rats between 16 – 2500 mg/kg bw). It can cause CNS depression (Lewis, 1996). According to the FEEDAP group, piperonal is an irritant to skin, eyes and respiratory tract (EFSA, 2012).

Because toxicological inhalation data on piperonal are missing, it is unclear what health effects are expected after exposure to piperonal from cigarette smoke. No data are available on outdoor or indoor inhalation exposure of the general population to piperonal.

Tobacco industry studies report that piperonal, even at exaggerated inclusion levels in cigarette tobacco compared with commercial inclusion levels, showed no toxicological sequelae. Importantly, comparative toxicity studies are performed for cigarette smoke with and without the additive of interest (Coggins *et al.*, 2011). Piperonal levels in the particulate fraction of several cigarettes smoked according to the FTC protocol contain <4-1010 ng piperonal (Stanfill & Ashley, 2000).

Pyrolysis experiments with 291 single-substance ingredients, including piperonal, were performed by the tobacco industry. Prior to these experiments, a set of pyrolysing conditions that approximates those occurring in the pyrolysis region of the burning cigarette were developed. The conditions included heating the sample at 30°C/sec from 300 to 900°C under a flow of 9 % oxygen in nitrogen. Pyrolysis of piperonal resulted in a pyrolysate containing 99.8 % piperonal. The remaining 0.2 % is probably methoxybenzoic acid (Baker *et al.*, 2004).

Addictiveness

Although piperonal is the precursor of MDMA and MDPV (3,4-methylene-dioxy-pyrovalerone), it is not psychotropic. It is unlikely that piperonal reacts to these compounds in tobacco smoke.

Piperonal has aromatherapeutic qualities that might elevate mood and well-being. Patients who were undergoing an MRI in an environment scented with heliotropin demonstrated a 63 % reduction in anxiety compared with those who were not exposed to fragrance (Redd *et al.*, 1994).

Research on brain effects by of additives by Philip Morris determined that the addition of vanillin, with a flavour similar to that of piperonal, increased P₁-N₂ amplitudes, but it is not clear what this means.

Characterising flavour

Piperonal is typically used as fragrance in amounts of 0.003-0.3 weight % (Opdyke, 2013), which is the same range that is typically added to tobacco products. Therefore, piperonal may lead to a noticeable flavour other than tobacco.

Rational for inclusion

Regarding attractiveness, piperonal is one of several aldehydes present in cigarette tobacco and cigarette smoke. Piperonal is a known flavouring agent for food and beverages and is added to tobacco products for flavouring. More data are needed on the amount of piperonal that imparts a noticeable flavour.

Regarding addictiveness, there are some studies indicating that piperonal has psychoactive effects, such as anxiety reduction. It is, therefore, suggested to perform fMRI studies on smokers and non-smokers, including adolescents who are exposed to piperonal, to study its effects on the brain.

To perform a toxicity risk evaluation, it is necessary to know the exposure level of piperonal through cigarette smoking. Therefore, research is needed to determine the amount of piperonal in mainstream cigarette smoke.

Because toxicological inhalation data on piperonal are missing, it is unclear what health effects are expected after exposure to piperonal from cigarette smoke. Such experiments should be carried out, particularly on the effects on CNS, irritation and sensitisation, as such effects were reported to occur.

It is unclear if toxic combustion products of piperonal are formed upon smoking a cigarette. Pyrolysis experiments performed by the tobacco industry indicate that piperonal transfers almost intact into the smoke, with some formation of methoxybenzoic acid. More studies on the combustion process of piperonal during smoking are needed.

3.3.24 Propylene glycol

Please refer to the PITOC Project factsheet

Rational for inclusion

Propylene glycol (PG) is added as humectant to tobacco, rather frequently and in relatively high amounts (1599 counts in NL ingredient lists, 23 in NTM, total number of brand 4265), average (weight %) 1.579 (1.636).

Regarding attractiveness, humectants are added to trap water, thereby keeping the moisture in the tobacco and preventing it from drying out. Internal tobacco industry documents reported that adding 3-7 weight percent of PG increased the mildness and reduced irritation (although this is higher than amounts typically present in tobacco cigarettes). Propylene glycol is, therefore, considered to positively influence the attractiveness of cigarette smoking given that humidification improves palatability of cigarettes. Propylene glycol does not have a strong flavour, and is, therefore, not expected to impart a noticeable flavour.

Regarding addictiveness, no data were reported to suggest that propylene glycol plays a role in smokers' addictiveness to cigarettes.

Regarding toxicity, it was reported by tobacco industry that the transfer rate of propylene glycol to mainstream smoke is 10 %. A risk assessment procedure using a Margin of Exposure (MOE) analysis concluded that risks of effects on the respiratory tract epithelium from propylene glycol exist. No thorough assessment on systemic effects was made.

Propylene oxide is regarded as possibly carcinogenic to humans and trace amounts are present in propylene glycol. Additionally, pyrolysis of propylene glycol results in formation of small amounts (<10 %) of 1,3-propylene glycol, acetol or acetic anhydride, and pyruvaldehyde.

Finally and importantly, propylene glycol and/or its combustion products is only one component out of the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur.

3.3.25 Sorbitol

Please refer to the PITOC Project factsheet

Rational for inclusion

Sorbitol is added as a humectant to tobacco (210 times in NL ingredient lists, 30 in NTM, total no of brands 4265), average (weight %) 0.232 (0.458).

Regarding attractiveness, humectants are added to trap water thereby, keeping the moisture in the tobacco and preventing it from drying out. Sorbitol is, therefore, considered to positively influence attractiveness of cigarette smoking given that humidification improves palatability of cigarettes. Sorbitol gives tobacco smoke a slightly bitter taste and a vague odour of cellulose and is, therefore, not expected to impart a noticeable attractive flavour when used in higher amounts.

Regarding addictiveness, no data were reported to suggest that sorbitol plays a role in smokers' addictiveness to cigarettes. However, its combustion products, such as acetaldehyde and formaldehyde, were proposed to increase the addictive effect of nicotine, although data on acetaldehyde produced by pyrolysis entering the brain through the smoke inhaled are inconclusive (SCENIHR 2010).

Regarding toxicity, sorbitol was reported to pyrolyse at 900°C to compounds, such as 2-furfural (31.4 %, see section on furfural), acetaldehyde (irritant and possible human carcinogen), formaldehyde (irritant, carcinogen). Other pyrolysis products of sorbitol

include furan, 2-methyltetrahydrofuran, propionaldehyde, acetone, methanol, and carbon monoxide (Baker and Bishop, 2004). Further research is needed to confirm these effects, especially if sorbitol pyrolysis results in carcinogenic compounds.

Finally, it must be borne in mind that sorbitol (and/or its combustion products) is only one component out of the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur.

3.3.26 Sugars

Please refer to the PITOC Project factsheet

Rational for inclusion

Many types of sugars are added, rather frequently and in relatively high amounts. Sugars are added as flavour or casing to tobacco (invert sugar is used most abundantly, with 767 counts in NL ingredient lists, none in NTM, total number of brands 4265), average (weight %) 2.734 (3.990).

Regarding attractiveness, the addition of sugars to tobacco was suggested to increase attractiveness by reducing the harshness of tobacco smoke caused by volatile basic components, such as ammonia, nicotine and other tobacco alkaloids. This is because upon cigarette smoking, sugars produce acids that reduce the pH of the inhaled smoke. In addition, the caramel flavours and the brown-coloured Maillard reaction products generated through the combustion of sugars in tobacco improve the taste and smell of tobacco products. More data are needed on the amount of sugars that impart a noticeable characterising flavour.

Regarding addictiveness data are inconclusive. Sugars in tobacco may act pro-addictively, because their combustion products such as acetaldehyde and formaldehyde have been suspected of increasing the addictive effect of nicotine. There are some studies indicating that sugars do not contribute to the production of acetaldehyde in mainstream smoke, on a weight-by-weight basis, greater than the overall formation of acetaldehyde from natural tobacco polysaccharides, including cellulose, which are the primary precursor of acetaldehyde in mainstream smoke (Cahours *et al.*, 2012). However, other studies performed in cigarettes with one type of tobacco showed that sugar content is positively correlated with the quantity of aldehydes produced. More research into the pro-addictive effects of sugars is warranted.

Regarding toxicity, only minor amounts of sugars (approximately 0.5 % of glucose and sucrose) are transferred unchanged into mainstream smoke, while the bulk of the sugar combusts or takes part in pyrosynthesis. Many pyrolysis products including organic acids, acetaldehyde (irritant and possible carcinogen), acrolein (irritant), 2-furfural (see section on furfural), acrylamide, and (carcinogenic) polyaromatic hydrocarbons (PAHs) were reported. Mainstream cigarette smoke with and without various levels of sugars was investigated in *in vitro* cytotoxicity and genotoxicity assays, *in vivo* inhalation toxicity studies with primary emphasis on irritative changes in the respiratory tract, and in dermal tumorigenicity studies. They did not show a significant increase of the parameters studied as marker of toxicity, but all of those studies had severe methodological and interpretation limitations (Roemer *et al.*, 2012).

Further research is needed to confirm the effects of combustion products of sugars, especially because they are possible or known carcinogenic compounds.

Finally and importantly, sugars and/or their combustion products are among the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur.

3.3.27 Titanium dioxide

General

CAS-nr: 13463-67-7 mixture of mainly rutile and anatase

CAS-nr: 1317-80-2 rutile

CAS-nr: 1317-70-0 anatase

Other names: titanium(IV) oxide, titania

Chemical structure: TiO_2

Titanium dioxide can be found in four mineral forms: rutile, anatase, brookite, akagooite.

Chemical class: metal oxides.

General use: Titanium dioxide is used as a white pigment, and has a wide range of applications including paint, sunscreen and food colouring. Titanium dioxide is an approved food-additive within Europe (E171) (anatase and rutile) (EFSA, AFC Panel, 2004).

Reported tobacco industry uses

The frequency of use in the NL list was 1329 (1256 in NTM), the average ingredient is 0.161 (% w/w) and is in the 6th position in the NL ranking (see Table 2).

Health effects

Toxicity

Given its use as food flavouring and in cosmetics, evaluations have focussed on oral and dermal exposure.

JECFA (1969) concluded that: "Titanium dioxide is a very insoluble compound. The studies in several species, including man, show neither significant absorption nor tissue storage following ingestion of titanium dioxide. Studies on soluble titanium compounds have therefore not been reviewed. Notably, following absorption of small amounts of titanium ions, no toxic effects were observed. Establishment of an acceptable daily intake for man is considered unnecessary."

Recently, there is much focus on the nano-form of titanium dioxide, which can also be with or without a coating.

The SCCS evaluated its use as cosmetic ingredient (sunscreen). With regard to inhalation toxicity, it was concluded that in subacute repeated dose inhalation toxicity studies, nano-size TiO_2 induce an acute inflammation in the lungs, that may be

reversible depending on the dose and the time after exposure. In view of this, acute inflammation (spray) applications, which may result in inhalation exposure, were not recommended by the SCCS (SCCS, 2014).

Both nano and non-nanosize titanium dioxide was classified by IARC as a Group 2B carcinogen (possibly carcinogenic to humans) (IARC, 2010).

Combustion products: not applicable because titanium is already in its highest oxidised state.

Addictiveness

No data.

Characterising flavour

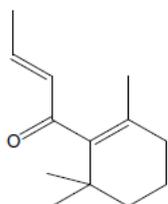
No data.

Rational for inclusion

To perform a risk evaluation, it is necessary to know the exposure level of titanium dioxide through cigarette smoking. Therefore, research is needed to determine the amount of titanium dioxide in mainstream cigarette smoke. Because inhalation toxicity is also related to the size of the particles, a distinction needs to be made between nano- and non-nano size.

3.3.28 Trimethyl (cyclohex-1-enyl)but-2-en-4-one (β -damascone)

General



CAS registry number: 23726-92-3.

cis- β -Damascone is a fragrance ingredient used in many fragrance compounds, with a typical rose scent. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents (Lalko *et al.*, 2007).

Molecular Weight: 192.2973 g/mol.

Molecular Formula: C₁₃H₂₀O

Reported tobacco industry uses

Cycloaliphatic unsaturated ketones, in general, are added as odour and taste-modifying agents in tobacco products (Kovats *et al.*, 1980).

β-Damascone is used up to 350 ppm (Baker *et al.*, 2004).

Health effects

Toxicity

In the one review found, low or no irritation or sensitisation was seen (Lalko *et al.*, 2007). Beta-damascone did not induce mutations in five strains of *Salmonella typhimurium*, (Bowen, 2011) beta-Damascone (purity: 95 %) was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system (Stone, 2012). The Panel concluded that beta-damascone is genotoxic in the *in vitro* micronucleus assay on human lymphocytes with metabolic activation and equivocal without metabolic activation. A combined *in vivo* micronucleus assay/liver Comet assay was performed after oral application of beta-damascone (purity: 95.6%) to further assess the genotoxic potential of beta-damascone and damascones more generally. The results from the combined *in vivo* micronucleus induction study and Comet assay show that orally-administered beta-damascone did not induce micronucleated erythrocytes in rat bone-marrow cells or genotoxic events in the liver and duodenum of rats.

The combined evidence from *in vitro* and *in vivo* genotoxicity data for beta-damascone does not indicate a genotoxic potential (EFSA opinion FGE213rev1, 2014).

Because for alpha-damascone and delta-damascone the available data could not rule out genotoxicity, it is crucial that the compound used is the beta-isomer and not the other two.

A modified Ames assay using the pre-incubation method was conducted in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA to assess the mutagenicity of delta-damascone (purity: 93.8 %). It was concluded that delta-damascone did not induce mutations in four strains of *S. typhimurium* or *E. coli* WP2uvrA under the conditions employed. The same mutagenicity Ames test carried out with alpha-damascone (purity: 96.9 %) and also produced negative results (Haddouk, 2001).

Three *in vitro* micronucleus experiments were performed using human peripheral blood lymphocytes to determine whether alpha-damascone was able to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system (Lloyd, 2012; Whitwell, 2012; Lloyd, 2013) giving equivocal results. Indeed, alpha-damascone induced statistically significant chromosomal damage or aneuploidy, but results were difficult to interpret due to the difficulty in assessing cytotoxicity. No *in vivo* tests are available: the current data available for alpha-damascone is not sufficient to exclude alpha-damascone genotoxicity (EFSA FGE210rev 1, 2014).

At 550 °C, only a small portion of the parent compound was pyrolysed. At 750°C, the compound was completely pyrolysed, with a conversion of 99.74 %, and 45 products were given. Moreover, with the elevation of the pyrolysis temperature, complex pyrolysis products were formed, and harmful substances, such as benzene, toluene, anthracene and phenanthrene were identified (Wu *et al.*, 2007).

Addictiveness

No data.

Characterising flavour

The compound is a flavour and used as such in food.

Rationale for inclusion

The compound *per se* is not considered to be toxic and does not have CMR properties. However, on the basis of the available data, potential for genotoxicity could not be ruled out for its isomers α - and δ -damascone. Therefore, the purity of β -damascone should be checked, to exclude the presence of the α - and δ - isomers. In addition, some data are available on the formation of various substances following combustion, namely benzene, toluene, anthracene and phenanthrene, which do have CMR properties.

3.3.29 Vanillin

Please refer to the PITOC Project factsheet

Rational for inclusion

Cigarette companies report using vanilla bean extract, vanillin and ethyl vanillin. Natural vanilla is a complex extract and expensive. Thus, synthetic vanilla flavour substances, vanillin and ethyl vanillin are used to reduce costs. Vanillin is one of the most universally accepted popular aromatic chemicals. Thermal decomposition or burning may release carbon monoxide or other hazardous gases, acrid smoke and irritating fumes. Vanilla flavour is one of the most popular flavours worldwide and is used to enhance the organoleptic properties (pertaining to taste, colour, odour, and touch, involving use of the sense organs) of tobacco smoke, to make the product more attractive to consumers, thereby, promoting and sustaining tobacco use, especially by young people and first-time users.

At low application levels, vanilla, the synthetic vanillin and ethyl vanillin are known to interact with other flavours and potentiate their effect, for example chocolate. Therefore, additional data is required to ascertain the effect of low vanillin levels and its interaction with other flavours on palatability and inhalation of smoke/nicotine.

3.3.30 Weak organic acids

Reported tobacco industry uses

Weak organic acids are used by tobacco industry mainly as flavouring substances in maximum levels in cigarettes between 0.0001 to 0.05 %. They are used also as

humectants. Some of them are added to the wrapper to control the puff number, ash appearance and reduce visible side stream.

Health effects

According to available data (e.g. OECD, JECFA, WHO, NTP reports/database) above mentioned, weak organic acids do not present a hazard for human health based on their low hazard profile. Most of them have GRAS status. After combustion, 80 – 100 % of these acids are found in the mainstream smoke in an intact form. They do not lead to the formation of compounds which have CMR properties in any of the products concerned (cigarettes/RYO) to a significant or measurable degree.

Addictiveness

No direct evidence found (see below).

Characterising flavour

Weak organic acids are added to tobacco during the manufacturing of cigarettes, to provide cigarettes with a distinct taste and smell. The rationale to use weak organic acids is linked also to their capability to regulate pH and to counter the effect of ammonia compounds. Therefore, they are suspected to facilitate the inhalation and absorption of nicotine, potentially resulting in increased addictiveness, although no conclusive data exist in this respect.

Rationale for inclusion

The rationale for inclusion of weak acids is linked to their capability to regulate pH and to counterpart the effect of ammonium compounds: indeed, the alkalinisation should be balanced, by adding also weak acids. Therefore, they are important for ease of smoking, facilitating inhalation and absorption of nicotine, resulting in increased attractiveness and addictiveness as well. To prioritise among this type of compounds, the more potent one should be examined first.

3.3.30.1 Citric acid

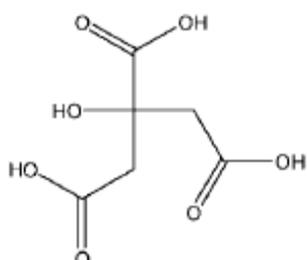
General

CAS Number 77-92-9: FEMA number: 2306.

Chemical class: Hydroxytricarboxylic acid.

Molecular weight: 192.

Boiling point: 142 C.



Reported tobacco industry uses

Citric acid is added as a flavouring substance and, among others, is added to the wrapper to reduce visible side stream.

In R.J. Reynolds and PMI lists of ingredients, max level used in cigarettes (flavour) is 0.0001%. The maximum recommended level of citric acid applied commercially to a cigarette is 11360 ppm (<https://www.rjrt.com/tobaccoingredients.aspx>; Baker *et al.* 2004).

Health effects

Toxicity

Citric acid was tested in a number of bacterial assays, all of which gave negative results. Evidence for genetic toxicity was described in a recent publication from an *in vitro* study. However, an *in vivo* chromosome aberration study did not support the conclusion of the recently reported *in vitro* studies in mammalian cells, and in an *in vivo* rodent dominant lethal assay there was no evidence of chromosome damage. Therefore, it is considered that the *in vitro* results do not reflect a potential for genetic toxicity and that a classification for mutagenicity is not required.

In a rat-feeding study, animals dosed with 5-% citric acid in the diet showed an excess of tumours in comparison with control animals when tested over a period of 2 years. However, there was some evidence that high doses of citrate salts potentiated the incidence of tumours produced by co-administration of known bladder carcinogens. When citric acid or citrate salts were administered alone during these studies, no dose-related tumours were noted. The conclusion is that citric acid is not carcinogenic in rats and mice of either sex (<http://toxnet.nlm.nih.gov/cpdb/pdfs/ChemicalTable.pdf>).

Reproductive toxicity: Various studies on rats, mice and guinea pigs using a number of different conditions and protocols including prior to mating, during pregnancy and a two-generation study were summarised in the OECD report (OECD, 2001). In some, the doses were defined and in others, the regimen was *ad libitum* feeding of a defined concentration of citric acid in the diet, with or without the measurement of food uptake. No adverse effects on females or foetuses were reported, except for slight dental attrition in the females in some of the studies. The NOEL values reported were often meaningless because only one dose was used, and that resulted in no adverse effects. In the same report described above, it was shown that 5 % citric acid in the diet of female mice and rats had no effect on the reproductive performance as measured by pregnancy rate, number of live births, still births and pup survival rate. In conclusion, citric acid has no effect on reproduction (<http://www.inchem.org/documents/sids/sids/77929.pd>).

The JEFCA evaluation concluded that citric acid and its calcium, potassium and sodium salts do not constitute a significant toxicological hazard to man (http://www.inchem.org/documents/jecfa/jecval/jec_436.htm).

Citric acid does not transform during pyrolysis (100%) (Baker and Bishop, 2004; Coggins *et al.*, 2011).

Addictiveness

No data found.

Characterising flavour

Citric acid, among others, is added to the wrapper to reduce visible side stream smoke components, and thus may contribute to attractiveness. (http://www.pmi.com/eng/our_products/pages/technical_products_information.aspx)

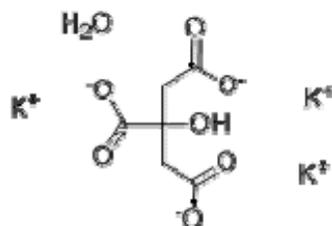
Information on minimum levels of odour awareness was not found.

3.3.30.2 Potassium citrate

General

CAS number 866-84-2; 6100-05-6

Molecular weight: 324.4



Chemical formula $K_3C_6H_5O_7 \cdot H_2O$

Boiling point: not available

Melting point: 180 C

Odour – odourless

Potassium citrate is an EU permitted Food Additive (E 332). Conditions of use: Quantum Satis. The US Food and Drug Administration classified potassium citrate as a GRAS (Generally Recognized As Safe) food ingredient.

Reported tobacco industry uses

Liu and Parry (2003) found that malate and citrate potassium could increase the puff number. Adding potassium as malate, citrate and tartrate salts to tobacco at a ca. 5% total level had similar overall effects per cigarette, which altered the ash morphology in a way that restricted the airflow and caused stronger bonding of the ash particles. It was recently confirmed in a study by Yin *et al.* (2014) in which they found that potassium salt in cigarettes significantly changed the morphology of ash during smoking, with melted potassium salt keeping it closely together.

Therefore, potassium citrate is added to the wrapper of the tobacco column to control puff number and ash appearance, which is important to be compliant with the maximum tar, nicotine and CO yields (Scollo and Winstanley, 2015).

Health effects

Toxicity

From information published on Toxnet Hazardous Substance Data Bank (<http://toxnet.nlm.nih.gov>), it is concluded the following for potassium citrate:

Inhalation: May cause mild – or no - irritation to the respiratory tract.

Ingestion: No adverse effects expected.

Skin Contact: May cause mild – or no - irritation and redness.

Eye Contact: Potassium citrate may cause mild irritation, possible reddening.

Chronic Exposure: No adverse health effects expected.

Aggravation of Pre-existing Conditions: No adverse health effects expected.

Addictiveness

No data found.

Characterising flavour

Potassium citrate is added to the wrapper of the tobacco column to control puff number and ash appearance, and thus may contribute to attractiveness.

3.3.30.3 Acetic acid

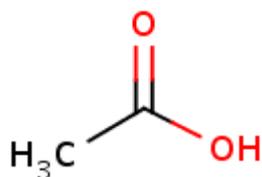
General

CAS Registry Number: 64-19-7; FEMA number 2006

Molecular Formula: C₂H₄O₂

Molecular weight: 60.05

Boiling point: 118 C



Acetic acid is a colourless liquid with a pungent, vinegary odour.

Reported tobacco industry uses

- Acetic acid is a chemical that has a distinct taste and smell. Vaporised acetic acid is just one of the many chemicals added to tobacco during the manufacturing of cigarettes in order to give cigarettes a distinct taste and smell.

- Relative amounts of acetic acid reported for various tobacco types were: Virginia 877 µg/g; Burley 372 µg/g; Oriental 688 µg/g. Its minimum detectable threshold level (flavour potency) was established as 22000 ppb (Kalianos, 1976).
- Acetic acid as an ingredients added to the tobacco presented by Philip Morris International (PMI) on cigarettes manufactured for sale in Germany has max use level (w/w %) of 0.05; max use level mg/cigarette – 700 mg – 0.07 %. In R.J. Reynolds list of ingredients max level used in cigarettes (flavour) is 0.001 %. The value of 500 ppm is on BAT list of additives (Baker *et al.* 2004; <https://www.rjrt.com/tobaccoingredients.aspx>; http://www.pmi.com/eng/our_products/pages/technical_products_information.aspx).
- The maximum recommended level of acetic acid applied commercially to a cigarette is 4500 ppm.

Health effects

Toxicity

Acetic acid was investigated for the respiratory tract irritation properties. The respiratory response and sensory irritation potential of acetic acid in Swiss Webster mice were investigated. Groups of eight mice were exposed for 30 minutes to eight acetic acid vapour concentrations ranging from 89 to 1730 ppm. Periocular wetness was observed in all animals following acetic acid exposures at 560, 572, 1694, and 1730 ppm. Other clinical signs noted immediately following exposure to 1694 and 1730 ppm were respiratory difficulty, decreased motor activity, and opacities of the eye. The concentration of acetic acid which produced a 50 % decrease in respiratory rate (RD50) was determined to be 577 ppm. (<http://industrydocuments.library.ucsf.edu/tobacco/docs/trdd0100>).

It was found that most of the acetic acid (95.9 %) is in mainstream smoke in the intact form. Maximum level in smoke may reach 2160 µg. Two other compounds have been identified as being formed during pyrolysis: acetic acid anhydride (4 %; max level in smoke- 90 µg and ethanol (0.1 %; max level in smoke 2 µg) (Baker and Bishop, 2004). In another study, 100 % of acetic acid was transferred to the mainstream smoke (Purkis *et al.* 2011). Thus, it does not form substances that may have CMR properties in the concentrations found in any of the products concerned (cigarettes/RYO).

Addictiveness

No data

Characterising flavour

It is added to tobacco during the manufacturing of cigarettes in order to give cigarettes a distinct taste and smell and thus may contribute to attractiveness.

3.3.30.4 Butyric acid

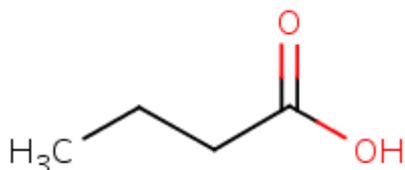
General

CAS Number: 107-92-6; FEMA Number: 2221

Chemical class: acid

Molecular weight: 88.11

Boiling point: 164 C



Reported tobacco industry uses

Butyric acid is added to tobacco during the manufacturing of cigarettes, to give cigarettes a distinct taste and smell.

Relative amounts of butyric acid reported for various tobacco types were: Virginia 2 µg/g; Burley 0 µg/g; Oriental 0 µg/g. Its minimum detectable threshold level (flavour potency) was established as 240 ppb (Kalianos, 1976).

Butyric acid as an ingredient added to the tobacco, based on a table presented by Philip Morris International (PMI) on cigarettes is 0.01 %. According to British American Tobacco maximum use is 0.00086 mg (0.0001 %) in the tobacco (Baker *et al.*, 2004).

According to the R.J. Reynolds list of ingredients, the maximum level used in any cigarette brand (flavour) is <0.001 % (<https://www.rjrt.com/tobaccoingredients.aspx>).

Health effects

Toxicity

According to the JEFCA report, there are no safety concerns at current levels of intake when used as a flavouring agent in food.

It does not lead to the formation of compounds, which may have CMR properties in any of the products (cigarettes/RYO) to a significant or measurable degree. During combustion, most of the compound (97.5 %) is not transformed. The maximum level in smoke is 54 µg. Two other unidentified substances were found at 2.5 % (maximum level in smoke 1.4 µg) (Baker and Bishop, 2004).

Addictiveness

No data found.

Characterising flavour

It is added to tobacco during the manufacturing of cigarettes in order to give cigarettes a distinct taste and smell and thus may contribute to attractiveness.

Its minimum detectable threshold level (flavour potency) was established as 240 ppb (Kalianos, 1976).

3.3.30.5 Lactic acid

General

CAS number: 50-21-5

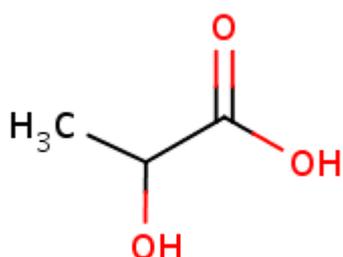
FEMA number: 2611

Chemical class: acid

Molecular formula: $\text{CH}_3\text{CH}(\text{OH})\text{CO}_2\text{H}$

Molecular weight: 90

Boiling point: 122 C



Reported tobacco industry uses

Lactic acid can keep tobacco humid, reduce the impurity of tobacco, improve the flavour and also offset the nicotine, reduce the elements that are harmful to human body and improve the quality of tobacco (http://www.china-musashino.com/pages/news4_en.htm).

Lactic acid, as an ingredient added to the tobacco presented by Philip Morris International (PMI) in cigarettes manufactured for sale in Germany, has the maximum use level (w/w %) of 0.05; the maximum use level mg/cigarette – 700 mg – 0.07 %. In the R.J. Reynolds list of ingredients, the maximum level used in cigarettes (flavour) is 0.001 %. The maximum cigarette level for lactic acid on BAT list of additives is 7100 ppm. (Baker *et al.* 2004; http://www.pmi.com/eng/our_products/pages/technical_products_information.aspx; <https://www.rjrt.com/tobaccoingredients.aspx>, <http://www.bat-ingredients.com>).

Health effects

Toxicity

Lactic acid is a natural, functional metabolite in mammals and serves as mammalian fuel. According to the "lactate shuttle" concept, it represents a major means of distributing carbohydrate potential energy for oxidation and gluconeogenesis.

According to OECD Report and JEFCA Report on lactic acid, it does not present a hazard for human health based on its low hazard profile (<http://apps.who.int/food-additives->

contaminants-jecfa-database/chemical.aspx?chemID=3367;
<http://webnet.oecd.org/HPV/UI/handler.axd?id=fd79fce6-c7e2-48ed-aead-8728c961980c>).

After combustion, lactic acid is found in mainstream smoke mostly in the intact form (83.2 %; maximum level in smoke – 5200 µg); other products of its pyrolysis that were found are: methylmaleic anhydride (3.7 %; maximum level in smoke 230 µg) and 2 lactides (13.1 %-810 µg) (Baker and Bishop, 2004; Baker *et al.*, 2004). Thus, it does not form substances that may have CMR properties in the concentrations found in any of the products concerned (cigarettes/RYO).

Addictiveness

Information has been found stating that lactic acid can offset the nicotine, however, no further details were given (http://www.china-musashino.com/pages/news4_en.htm).

Characterising flavour

As a flavouring substance may increase attractiveness.

3.3.30.6 2-methyl butyric acid

General

CAS number: 116-53-0

FEMA number: 2693

Chemical class: acid

Molecular weight: 102

Boiling point: 176 C

Reported tobacco industry uses

- 2-methyl butyric acid is added to tobacco as flavouring agent (cranberry aroma).
- 2-methyl butyric acid as an ingredient added to the tobacco presented by Philip Morris International (PMI) in cigarettes manufactured for sale in Germany has max use level (w/w %) of 0.0001. In the R.J. Reynolds list of ingredients, the maximum level used in cigarettes (flavour) is 0.001 %. The maximum cigarette level of 2-methylbutyric acid, on BAT list of additives is 7.5 ppm (Baker *et al.*, 2004; http://www.pmi.com/eng/our_products/pages/technical_products_information.aspx; <https://www.rjrt.com/tobaccoingredients.aspx>; <http://www.bat-ingredients.com>).
- Amounts of 2-methyl butyric acid reported for various tobacco types were: Virginia 247 µg/g; Burley 26 µg/g; Oriental 313 µg/g. Its minimum detectable threshold level (flavour potency) was established as 1600 ppb (Kalianos, 1976).

Health effects

Toxicity

According to the OECD Report and the JEFCA Report on 12-methyl butyric acid, it does not present a hazard for human health based on its low hazard profile (<http://apps.who.int/food-additives-contaminants-jecfa->

database/chemical.aspx?chemID=3367,
<http://webnet.oecd.org/HPV/UI/handler.axd?id=fd79fce6-c7e2-48ed-aead-8728c961980c>).

After combustion, 2-methylbutyric acid is found in the mainstream smoke mostly in the intact form – 89.6 % maximum level in smoke (3 µg). Other compounds found there were: propanoic acid – 1,4 % (0.05 µg); amyl isovalerate 0.3 % (0.01 µg) and other not fully or unidentified substances – 2.1 % (Baker and Bishop, 2004).

Thus, it does not form substances that may have CMR properties in the concentrations found in any of the products concerned (cigarettes/RYO).

Addictiveness

No data found.

Characterising flavour

As a flavouring substance may contribute to attractiveness.

3.3.30.7 Sorbic acid

General

Sorbic acid and its salt potassium sorbate are antimicrobial agents used as preservatives predominantly to prevent the growth of filamentous fungi and yeast frequently used to maintain the freshness of a variety of foods, drugs, and cosmetic products. Sorbic acid and potassium sorbate are approved as food additives for use as preservatives in a wide range of commonly consumed foods and are authorised as preservatives in feed for all animal species without restriction (EFSA, FEEDAP, 2014).

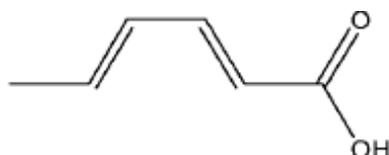
CAS number: 110-44-1

Chemical class- unsaturated acid

Molecular weight – 112

Melting point – 133 C

Boiling point – 228 C.



Reported tobacco industry uses

Sorbic acid is used by tobacco industry as a flavouring substance. The maximum recommended level of sorbic acid applied commercially to a cigarette is 0.1 ppm (Baker *et al.*, 2004).

Health effects

Sorbic acid is readily metabolised. Both humans and rats appear to utilise identical metabolic mechanisms for oxidation of sorbate. The long-term studies suggest that the same no-effect level applies to the salts as to the free acid. Sorbic acid and K-sorbate do not cause tumours when administered orally or subcutaneously. (http://www.inchem.org/documents/jecfa/jecval/jec_2181.htm)

In the NIH Carcinogenic Potency Database, it was not found to be carcinogenic in male rats and mice. (<http://toxnet.nlm.nih.gov/cpdb/chempages/SORBIC%20ACID.html>)

Most of the sorbic acid (99.4 %) is in mainstream smoke in the intact form. Maximum levels in smoke may reach 0.05 µg. Two other compounds identified during pyrolysis are: phenol (0.5 % - 0.0003 µg) and decanal 0.1 % - 0.0005 µg) (Baker and Bishop 2004).

Other sources (Purkis *et al.*, 2011) demonstrated that during pyrolysis only 68.6 % of sorbic acid is transferred intact to mainstream smoke; 6 other chemicals (>1 %) were also found, but details were not provided in the study.

Addictiveness

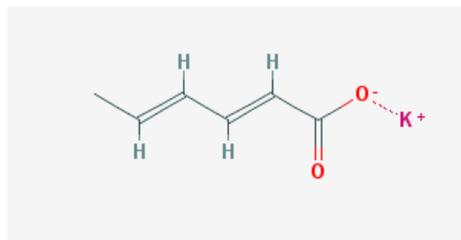
No data found.

Characterising flavour

As a flavouring substance may contribute to attractiveness.

3.3.30.8 Potassium sorbate

General



CASRN: 590-00-1.

Potassium sorbate (PS) is added to food with a wide range of moisture content as a preservative. Similar preservative effects can be expected in semi-moist feed for dogs and cats.

Reported tobacco industry uses

Potassium sorbate (PS) may be incorporated in blended cigarette tobacco either as a mould growth inhibitor in processed tobacco sheet material, or as a preservative in flavour systems or paper adhesives (Gaworski *et al.*, 2008).

Used up to 500 ppm (Baker *et al.*, 2004).

Pipe tobaccos contain humectants (e.g., glycerol and propylene glycol), preservatives (e.g., sodium benzoate, potassium sorbate) - 1.92 g potassium sorbate/kg in waterpipe tobacco (Thomsen *et al.*, 2010).

Health effects

Toxicity

In all *in vitro* and *in vivo* tests, no signs of genotoxicity were detected (Münzner *et al.*, 1990, Mpountoukas *et al.*, 2008).

As reported in Toxnet (May 2015): Can cause eye irritation.

At simulated tobacco burning temperatures up to 1000°C, neat potassium sorbate completely pyrolyzed to form aromatic ring materials including benzene, toluene, propyltoluene, xylene, methylxylene, styrene, phenol and butanone. Biological studies indicated that there were no relevant differences in the genotoxic or cytotoxic potential of either mainstream smoke from cigarettes with or without added potassium sorbate. Rats exposed to mainstream cigarette smoke developed respiratory tract changes consistent with those seen in previous smoke inhalation studies, with no relevant histopathological differences between the control and the potassium sorbate test cigarette groups. These studies demonstrated that high levels of potassium sorbate could alter the burning rate of the tobacco leading to alteration in the smoke chemistry profile. Yet, based on the panel of biological endpoints monitored, it appeared that added potassium sorbate produced little relevant change in the overall toxicity profile of smoke (Gaworski *et al.*, 2008).

Addictiveness

No data found.

Characterising flavour

No data found.

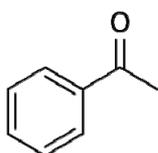
3.4 Additional substances

3.4.1 Acetophenone

General

This colourless, viscous liquid is a precursor to useful resins and fragrance, and a material for the synthesis of some pharmaceuticals. Acetophenone is an ingredient in fragrances that resembles almond, cherry, honeysuckle, jasmine, and strawberry. Acetophenone occurs naturally in many foods including apples, cheese, apricots, bananas, beef, and cauliflower. It is also a component of castoreum, the exudate from the castor sacs of the mature beaver.

CAS 98-86-2, aromatic ketone.



Reported tobacco industry uses

Added as flavour to tobacco (352 counts, none in NTM), average (weight %) 0.0014 (0.006).

Health effects

GRAS status. Note that this is not sufficient proof of safety as tobacco additive because the component is inhaled not ingested and combustion products may be toxic.

Toxicity

The LD₅₀ is 815 mg/kg (oral, rats). Acetophenone is currently listed as a Group D carcinogen (Not Classifiable as to Human Carcinogenicity), indicating that it does not produce carcinogenic effects in humans, although no studies on humans have ever been conducted on acetophenone carcinogenic potential.

There was no evidence of mutagenicity in Ames bacterial tests. Chromosome damage was reported in hamster cells in culture after metabolic activation (BIBRA working group, 1991 and EFSA AFC Panel, 2008).

EFSA decided that there is some indication of genotoxic potential for two of the supporting substances (1-phenylethan-1-ol and acetophenone). However, in the light that 1-phenylethan-1-ol can be metabolised to acetophenone and vice versa and that the results of a carcinogenicity study with 1-phenylethan-1-ol in mice and rats did not give rise to concern, the Panel concluded that the positive *in vitro* results for the two supporting substances did not raise concerns with respect to carcinogenicity in humans.

The U.S. EPA evaluated the non-cancer oral data for acetophenone and derived a reference dose (RfD) of 0.1 mg/kg-day based on general toxicity in rats.

When heated to decomposition it emits acrid smoke and fumes (Lewis, 1996).

Addictiveness

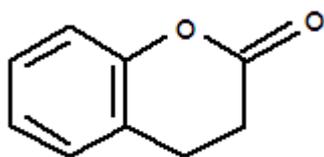
The SCENIHR report mentioned that acetophenone may have an effect on the CNS, referring to an internal RJ Reynolds document: <http://legacy.library.ucsf.edu/tid/sqp95d00/pdf>. This report refers to a hypnotic effect. It is known that in the late 19th and early 20th centuries, acetophenone was used as a medicine. It was marketed as a hypnotic and anticonvulsant under brand name Hypnone. The typical dosage was 0.12 to 0.3 millilitres. As the density is approximately 1 g/ml, this amounts to a few grams, which is not relevant given that this is orders of magnitude higher than the dose intake via cigarettes.

Characterising flavour

Typically used as fragrance in amounts of 0.002-0.09 weight % (Monographs on Fragrance Raw Materials, 2013).

This is the same range as added to tobacco products. Therefore, acetophenone may lead to a noticeable flavour other than tobacco.

3.4.2 3,4-Dihydrocoumarin



General

CAS Number 119-84-6.

White to pale yellow clear oily liquid with a sweet herbal odour and taste. Solidifies at approximately room temperature (Pubchem). The compound was evaluated by JECFA as well as by two EFSA Panels (EFSA CEF Panel, 2009; EFSA FEEDAP Panel 2012).

Health effects

Toxicity

The oral LD₅₀ in rats is 1460 mg/kg and in guinea pigs is 1760 mg/kg, indicating a low acute toxicity.

The safety data sheets identified the compound as irritant to skin, eye and the respiratory tract and harmful if swallowed. In addition 3,4-dihydrocoumarin was shown to be a skin sensitiser. These data are also reported by Pubchem.

For 3,4-dihydrocoumarin, the NOAEL identified by JECFA was 300 mg/kg bw from a two-year study in mice and rats (by gavage, based on increased incidences of renal tubular adenomas and focal hyperplasia in male F344/N rats, NTP, 1993). 3,4-dihydrocoumarin

was negative in bacterial tests for mutagenicity and in four studies of chromosomal aberrations in CHO cells *in vitro*. In an assay in mouse lymphoma cells, positive results were obtained in the presence of metabolic activation from S9. However, 3,4-dihydrocoumarin did not induce micronuclei in mouse peripheral blood cells *in vivo*.

3,4-dihydrocoumarin, when tested for long-term toxicity and carcinogenicity in mice and rats, did not increase neoplasms relevant for the safety evaluation in humans (JECFA, 2004b; NTP, 1993).

Dihydrocoumarin, a metabolite of coumarin, is hydrolysed to the corresponding ring-opened hydroxycarboxylic acid. The hydrolysis product may undergo either (i) β -oxidation and cleavage to yield o-hydroxybenzoic acid (salicylic acid), which is excreted primarily in the urine unchanged, or (ii) glycine conjugation followed by excretion primarily in the urine (JECFA, 2004). As this compound is devoid of the 3,4 double bond present in coumarin, epoxidation cannot occur and consequently metabolites of toxicological concern are not generated (Born *et al.*, 1997). 3,4-dihydrocoumarin does not accumulate to a significant extent in any tissue following oral administration to the rat and rabbit (Kaighen and Williams, 1961; Feuer *et al.*, 1966).

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the safety and efficacy of alicyclic and aromatic lactones (chemical group 11) when used as flavourings for all animal species. (EFSA, FEEDAP Panel, 2012).

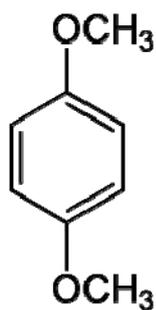
3.4.3 Dimethoxybenzene

General

CAS number: 150-78-7

Molecular weight: 136

Chemical formula: C₈H₁₀O₂



Boiling point: 213°C.

Melting point: 180 C.

Odour – intensive sweet floral odour.

FEMA: 2585.

It is produced by several plant species such as willow, tea, hyacinth. Dimethoxybenzene is mainly used in perfumes and soaps. The US Food and Drug Administration classifies dimethoxybenzene as a GRAS (Generally Recognized As Safe) food ingredient.

JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances". The Council of Europe Committee of Experts on Flavouring Substances proposed a practical upper level for foods of 5mg/kg in its advisory list.

Reported tobacco industry uses

The maximum recommended level of dimethoxybenzene applied commercially to a cigarette as a flavouring agent is 0.0001.

Health effects

Toxicity

Dimethoxybenzene in concentrations used in industry has irritant properties to skin and eyes and by inhalation. It is not classifiable as to its CMR properties to humans.

Dimethoxybenzene was identified as the major chemical in musk willow (*Salix aegyptiaca* - SA) extracts. In Iranian traditional medicine, SA was employed as a laxative, cardioprotective, nervonic, sedative, hypnotic, somnolent, aphrodisiac, orexiogenic, carminative, gastroprotectant, anthelmintic and vermifuge. The decoction of SA's leaves in honey is still used as a functional food. The decoction of leaves of SA plus sugar was used among Iranian and Turkish people for maladies like depression, neuropathic pain and rheumatoid arthritis.

Dimethoxybenzene is a carbonic anhydrase (CA, EC 4.2.1.1) inhibitor (CAI), interacting with the CA isozymes I, II (cytosolic) and IX, XII (transmembrane, tumour-associated).

It does not transform during pyrolysis (Purkis *et al.*, 2011).

Addictiveness

No data found.

Characterising flavour

Dimethoxybenzene is added to tobacco as a flavouring agent and thus may contribute to attractiveness. More data are needed on the amount of Dimethoxybenzene that potentially imparts a noticeable flavour.

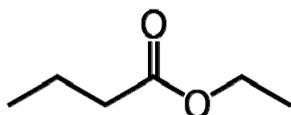
3.4.4 Ethylbutyrate

General

Ethyl butyrate has a fruity odour, similar to pineapple. It is commonly used as an artificial flavouring resembling orange juice or pineapple in alcoholic beverages (e.g. martinis, daiquiris, etc.), as a solvent in perfumery products, and as a plasticiser for cellulose. In addition, ethyl butyrate is often added to orange juice, because most people associate its odour with that of fresh orange juice.

Ethyl butyrate is one of the most common chemicals used in flavours and fragrances. It can be used in a variety of flavours: orange (most common), cherry, pineapple, mango, guava, bubble-gum, peach, apricot, fig and plum. In industrial use, it is also one of the cheapest chemicals, which adds to its popularity.

CAS: 105-54-4, ester.



Reported tobacco industry uses

Added as flavour to tobacco (306 counts, none in NTM), average (weight %) 0.046 (0.21).

Health effects

JECFA: ADI 0-15 mg/kg. Note that this is not sufficient proof of safety as a tobacco additive, because the component is inhaled not ingested, and combustion products may be toxic.

Toxicity

LD₅₀ oral: Rat 13,050 mg/kg; Rabbit 5.2 g/kg. In humans, it is a mucous membrane irritant and CNS depressant in high concentrations. No sensitisation processes after a 48 hour closed-patch test in 25 human subjects (ToxNet).

Addictiveness

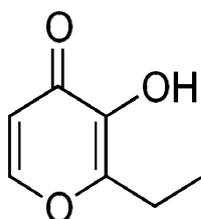
No reports.

Characterising flavour

Typically used as fragrance in amounts of 0.005-0.01 weight % (Monographs on Fragrance Raw Materials, 2013). Even more than this amount of ethylbutyrate is generally added to tobacco products. Ethylbutyrate may impart a noticeable flavour other than tobacco.

3.4.5 Ethyl maltol

General



CAS-nr: 4940-11-8

Other names: 2-Ethyl pyromeconic acid, 2-ethyl-3-hydroxy-4-pyrone

Chemical structure:

Chemical class: pyranones

Ethyl maltol is an approved food additive within Europe (E637), synthesised from maltol, and is used as a synthetic flavouring.

Reported tobacco industry uses

Ethyl maltol is added to tobacco cigarettes for its flavouring properties in amounts of 0.01 % w/w (RJRT).

Health effects

Toxicity

Ethyl maltol was evaluated by the JECFA (1974). Given the use as a food additive, the focus was on oral exposure. Short-term studies in rat and dog indicated no abnormalities upon repeated oral exposure (90 days). A long-term exposure study in rat also did not indicate any treatment-related effect reference. Results of a one-generation study did not reveal effects on parental animals or offspring (JECFA (1974)).

EFSA also evaluated ethylmaltol in the FGE 213 and concluded that the genotoxicity concern for ethylmaltol could be excluded based on two *in vitro* and one *in vivo* studies: in addition, a chronic study was available in which groups of 25 male and female rats were fed for two years on diets containing ethyl maltol calculated to deliver 0, 50, 100 and 200 mg ethyl maltol/kg bw/day. No abnormalities were seen for survival, clinical appearance, growth rate or food consumption, clinical chemistry, haematology and urinalysis. No histopathological changes and no increases in neoplasms were seen after treatment with ethyl maltol, thus, concluding that ethylmaltol is not carcinogenic via the oral route (EFSA, 2014b).

Tobacco smoke from test cigarettes containing ethyl maltol and additive-free reference cigarettes were tested in 90-day nose only inhalation studies with rats. In these studies, the biological activity of the smoke was not altered by adding ethyl maltol (Vanscheeuwijck *et al.*, 2002; Renne *et al.*, 2006).

No other information was found regarding relevant studies on inhalation exposure.

Intact transfer rates of close to 100 % have been reported from pyrolysis studies (Baker and Bishop, 2004).

Addictiveness

N/A.

Characterising flavour

No information on minimum levels of odour awareness was found for ethyl maltol.

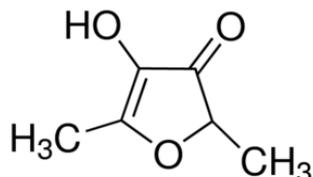
3.4.6 4-hydroxy-2,5-dimethyl-3(2H)-furanone

General

CAS-nr: 3658-77-3

Other names: furaneol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone

Chemical structure:



Chemical class: furans

General use: 4-hydroxy-2,5-dimethyl-3(2H)-furanone is used as a flavouring (strawberry flavour).

Reported tobacco industry uses

4-hydroxy-2,5-dimethyl-3(2H)-furanone is added to tobacco cigarettes for its flavouring properties in amounts of up to 0.01 % w/w (RJRT).

Health effects

Toxicity

4-hydroxy-2,5-dimethyl-3(2H)-furanone was evaluated by the JECFA (2005). Given the use as a food additive, the focus was on oral exposure: Genotoxicity was observed for this furan, though this was considered to be an effect caused by high dose and related to a mechanism involving reactive oxygen species, rather than the generation of a reactive metabolite, such as an epoxide. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (No 1446) showed no carcinogenicity in a 2-year study in which rats were given a dose of up to 400 mg/kg bw per day. For 4-hydroxy-2,5-dimethyl-3(2H)-furanone, the NOEL of 200 mg/kg bw/d from a 2-year dietary study in rats is >2300 times the estimated daily per capita intake of this agent from its use as a flavouring agent in Europe or the USA. The Committee, therefore, concluded that the safety of this agent would not be a concern at the estimated current intake. This was recently confirmed by EFSA (2015).

No other international reviews were found (i.e., IPSC, US EPA, HC, etc.), including studies on inhalation exposure.

Tobacco smoke from test cigarettes containing 4-hydroxy-2,5-dimethyl-3(2H)-furanone I and additive-free reference cigarettes were tested in 90-day nose-only inhalation studies with rats. In these studies, the biological activity of the smoke was not altered by adding ethyl maltol (Vanschreeuwijck *et al.*, 2002; Renne *et al.* 2006).

A pyrolysis study investigated the composition of the pyrolysate and reported 70.1 % acetic acid, 25.4 % acetic anhydride, 1.8 % acetol acetate, and 0.4 % benzaldehyde (Baker and Bishop, 2004).

Addictiveness

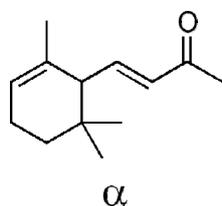
No data.

Characterising flavour

No information on minimum levels of odour awareness was found for 4-hydroxy-2,5-dimethyl-3(2H)-furanone.

3.4.7 Ionone (mixed alpha and beta isomers)

CAS Number: 8013-90-9.



$C_{13}H_{20}O$

Alpha ionone CAS Number: 127-41-3 30685-95-1

Beta-ionone CAS Number 14901-07-6 (alternate)

Ionones are aroma compounds found in a variety of essential oils, including rose oil (particularly β -Ionone). They are colourless to light yellow liquids used in perfumery. The combination of α -ionone and β -ionone is characteristic of the scent of violets and used with other components in perfumery and a flavouring to recreate their scent. The ionones are derived from the degradation of carotenoids.

In FGE210 (2008), EFSA considered that the genotoxicity concern for alpha-ionone could not be ruled out based on the genotoxicity data and (Quantitative) Structure-Activity Relationship ((Q)SAR) predictions available. In its revised opinion (FGE.210Revision1) issued in 2014 (EFSA, 2014b), additional genotoxicity data submitted by the industry were evaluated and the genotoxic potential for alpha-ionone was ruled out. It induced neither gene mutation in *S. typhimurium* nor structural or numerical chromosomal aberrations when tested with human peripheral lymphocytes. Alpha-ionone was tested also in an *in vivo* mouse bone marrow micronucleus assay in which no statistically significant increase in the frequency of micronucleated cells was observed. The result is considered to be reliable because there was an indication for bone marrow exposure (EFSA, 2014b).

In FGE213 (2009), EFSA considered that the genotoxicity concern for beta-ionone could not be ruled out based on genotoxicity data and the (Quantitative) Structure-Activity Relationship ((Q)SAR) predictions available. In its revised form (FGE.213 Revision1), additional genotoxicity data submitted by the food additives industry were evaluated, and concern for the genotoxic potential was excluded for beta-Ionone. Beta-ionone was

tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the absence and presence of S9-mix, resulting in an absence of mutagenic effect (Ballantyne, 2011, cited in EFSA, 2014b). Beta-ionone was also evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat liver S9-mix fraction as an *in vitro* metabolising system and the results were also negative. The combined evidence from *in vitro* and *in vivo* genotoxicity data on beta-ionone indicates a lack of genotoxic potential (EFSA, CEF Panel, 2014b).

Toxicity

In addition to the EFSA Opinion, ionone was also evaluated by SCCS in 2012: information was taken from these documents and from Lalko *et al.* (2007a).

Carotenoide breakdown products *in vitro* strongly inhibit state 3 respiration of rat liver mitochondria at concentrations between 0.5 and 20 μM . This was true for retinal, β -ionone and for mixtures of cleavage/breakdown products. The inhibition of mitochondrial respiration was accompanied by a reduction in protein sulfhydryl content, decreasing GSH levels and redox state, and elevated accumulation of malondialdehyde (Siems *et al.*, 2009).

Dietary beta-ionone demonstrates anti-cancer activity both *in vitro* and *in vivo* (Liu *et al.*, 2008).

Experimental data indicate that around 91 % of α -ionone and 95 % of β -ionone should transfer to the smoke-stream intact (Baker and Bishop, 2004).

Mixture of isomers:

Acute toxicity

Rat Oral: The LD50 was calculated to be 4.6 g/kg (95 % CI 3.9–5.4 g/kg). Major toxic signs included depression and tremors (Jenner *et al.*, 1964).

Mice Oral: LD50 was reported to be 10 g/kg (RIFM, 1980). Clinical signs included stress, laboured breathing, uncoordinated movement, hypothermia, lacrimation and bloated stomach.

Mice i.p.: LD50 was calculated to be 2.3 g/kg.

Mice s.c.: The LD50 was calculated to be 2.6 g/kg (95 % CI 2.1–3.2 g/kg)

Skin irritation

Human studies: (24 hour closed patch test) and (24-72 hour closed patch test) in adult volunteers of undiluted and 2-20 % ionone: No irritation was observed.

Animal studies: In a 4-hour semi-occlusive patch test in rabbits, irritation was observed in all animals (RIFM, 1979). In rats, slight erythema and oedema were observed at all concentrations in all animals (RIFM, 1981).

Skin sensitisation: Ionone did not produce sensitisation reactions in humans (Greif, 1967) or in animals.

A phototoxicity test was conducted in rats: no phototoxicity was observed (RIFM, 1980).

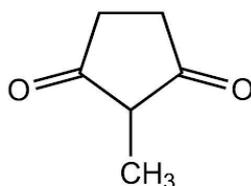
Subchronic toxicity

Osborne-Mendel rats were given ionone (60 % alpha, 40 % beta) in the diet at 1000 ppm, 2500 ppm, or 10,000 ppm for 17 weeks (approximately equivalent to 50, 125, and 500 mg/kg body weight/day). Histopathology examination of the liver, revealed slight to moderate swelling of hepatocytes in high-dose animals, slight swelling in mid-dose animals which the authors interpreted as an adaptive response rather than an “adverse” effect. No other effects were observed (Bar and Griepentrog, 1967, cited in Lalko *et al.*, 2007a, Carmines, 2002). Other studies did not evidence any alteration in hepatotoxicity markers or other enzymes (Sporn and Dinu, 1964, cited in Lalko *et al.*, 2007a).

The effects of ionone on reproduction of rats, given 2 mg ionone in 0.1 ml oil solution on alternate days for 8 months (equivalent to a dose of approximately 8–10 mg/kg body weight/day), was studied: Ionone had no adverse effect on any of the parameters measured. No effects were observed for ionone at approximately 10 mg/kg body weight/day (Belsito *et al.*, 2007, cited in Lalko *et al.*, 2007a).

3.4.8 3-Methyl cyclopentane-1,2-dione

General



CAS No. 765-70-8

It is also called maple lactone and is used as a flavouring substance having a maple-syrup caramel flavour, woody and sweet aromatic fruity taste. Maple lactone is used in cockroach attractant traps placed in dark or humid indoor areas where cockroaches are usually found. The use of these traps is not expected to have adverse health effects in humans or pets.

Based on reviews of the available toxicology data and other information related to maple lactone, the EPA found that this active ingredient is not likely to produce adverse health effects in humans.

3-methylcyclopentane-1,2-dione was evaluated by the Joint FAO/WHO Expert Committee on Food Additives.

85% of 3-methyl cyclopentane-1,2-dione should transfer to the smoke-stream intact (Baker and Bishop, 2004).

4 OPINION

The Tobacco Products Directive 2014/40/EU in its Article 6 calls upon the Commission to develop and update a priority list of at least 15 additives contained in cigarettes and roll-your-own tobacco by May 2016. The main purpose of this scientific Opinion is to assist the Commission to identify the additives that should be put on the priority list.

The SCENIHR was asked to identify those additives amongst the most commonly used additives by weight or number that have one or more of the following attributes:

- a. Contributing to the toxicity/addictiveness of the products concerned and/or increases the toxicity/addictiveness of any of the products concerned (cigarettes/Roll-Your-Own) to a significant or measurable degree;
- b. Resulting in a characterising flavour;
- c. Facilitating inhalation or nicotine uptake;
- d. Leading to the formation of substances that have CMR properties and/or increasing the CMR properties in any of the products concerned (cigarettes/roll-your-own) to a significant or measurable degree.

Some additives may fall into several of the above-mentioned categories. Specific additives can have a typical toxicological profile in their unburnt form; interactions between additives and of additives with other constituents of tobacco can also occur, as the tobacco product is a complex mixture, leading to the formation of other chemicals or increasing the toxicity of the mixture. In addition- and most importantly- combustion of tobacco generates substances that may be toxic and often show CMR properties. A good example is provided by aldehydes, such as formaldehyde, acetaldehyde, propanal, 2-butenal, 2-methylpropenal, butanal, methylbutanal, furfural, benzaldehyde, methylfurfural and methoxybenzaldehyde (Adam *et al.*, 2006, Baker *et al.*, 2004), formed by the pyrolysis of various sugars and polysaccharides that are both present as natural components of tobacco as well as added to tobacco products.

Increased nicotine bioavailability (obtained e.g. by adding chemicals that alter the pH of tobacco) and facilitation of tobacco smoke inhalation (possibly associated with additives with local anaesthetic effects, bronchodilators and humectants) can contribute to addictiveness of tobacco products. Among the many factors influencing attractiveness (including marketing actions intended to reduce concerns, e.g., with "light" branding), a particularly relevant factor is the generation of product sensory characteristics, e.g., by using additives resulting in characterising flavours. The flavours (especially sweeteners) are added to the natural tobacco to provide a better taste, which may increase the attractiveness of these products and maintain a specific and standardised taste of the product, which makes it unique and recognisable among the large variety of available brands.

To compile the list of priority substances, the SCENIHR considered data reported by the tobacco industry in the context of ingredient reporting under Directive 2001/37/EC. Some of these lists of additives were also published by Member States such as

Belgium¹⁶, the Czech Republic¹⁷, Germany¹⁸ and the Netherlands¹⁹. An additional list was received from the UK authorities when the work for drafting the Opinion was in an advanced stage; some were taken from other jurisdictions (e.g., USA, Canada, and Brazil) as well as from data published by industry²⁰. For practical reasons (not having available an EU-wide list), the comprehensive list from the Netherlands containing 1260 compounds was used as a typical example as verified in light of data submitted by other Member States. Most of the additives selected in the priority list belong to those frequently used in the other available sources. Therefore data on 'use and frequency' were considered to be sufficiently representative of the data on EU-wide basis.

The representativeness of data on 'use and frequency' on the EU-wide basis has been verified by DG SANTE by comparison with other industry submissions.

No information about single specific additives other than the most used ones was obtained from the analysis of papers from the literature search. Therefore, the NL list provided a representative basis for the selection which was carried out by applying the following inclusion criteria: amount and frequency of use, toxicity (including CMR properties) of the additive in its unburnt form or via the formation of toxic substances after combustion, information on the possibility of resulting in a characterizing flavour (one of the factors possibly contributing to attractiveness), and/or of facilitating inhalation and increasing nicotine uptake (possibly contributing to addictiveness).

Additives were ranked according to the frequency of detection in different brands as well as by the highest amount used in tobacco products (expressed as % w/w), which were considered as criteria for selection. To identify the substances for the priority list, a first cut-off for frequency in a brand was used, obtaining approximately 100 compounds.

After an initial scan, keeping in view the points in the article 6, 2 a-b, focussing on those additives present in tobacco and papers and excluding those mainly used in non-combusted components (e.g., filters), the SCENIHR ended up with a total of 55 chemicals for which a literature search for data on general characteristics of the compounds, toxicity data (including CMR properties) in the unburnt form, information about properties resulting in a characterising flavour (one of the factors potentially contributing to attractiveness), facilitating inhalation or increasing nicotine uptake (potentially contributing to addictiveness of the tobacco products), data on pyrolysis products and their toxicity was carried out. This method allowed some priority additives to be identified mainly based on hazard identification and it is not a thorough toxicological evaluation of the large number of selected compounds.

Although the SCENIHR was aware of the work and the outcome of the EU project "Public Information Tobacco Control" (PITOC), these compounds were not included on the priority list *a priori*. An independent selection on the basis of the agreed inclusion/exclusion criteria was carried out, regardless of the PITOC results. After the first screening, it appeared that on the list of the 55 chemicals selected by the SCENIHR,

¹⁶ <http://www.health.belgium.be/eportal/Myhealth/Tobacco/Fabrication/Database/index.htm>

¹⁷ <http://www.szpi.gov.cz/lstDoc.aspx?nid=11323>

¹⁸ http://www.bmel.de/DE/Ernaehrung/Gesundheit/NichtRauchen/_Texte/Tabakzusatzstoffe.html

¹⁹ <http://www.rivm.nl/Onderwerpen/T/Tabak>

²⁰ <http://www.bat-ingredients.com/>

those that had already been selected and evaluated by the PITOC project were included (Ammonium compounds, Carob bean extract, Cellulose, Cocoa, 2-furfural, Glycerol, Guar gum, Liquorice, Menthol, Propylene glycol, Prune juice concentrate, Sorbitol, Sugars, Vanillin), except for Carob bean extract, which was *a posteriori* added to the list, which included a total of 56 additives.

The report prepared by DKFZ (DKFZ, 2012) and the Annex for the factsheets for professionals that were created during this project by RIVM in the context of the project (RIVM, 2012) are attached as Annex 1.

For those additives already evaluated by the PITOC Project, only an update of the literature (starting from 2012) was performed, because the toxicological properties, as well as factors contributing to addictiveness and attractiveness characteristics, were already summarised in the data sheets prepared by the PITOC project. No new relevant information is available that would require an adaptation or change in the conclusions of the PITOC report. The characteristics on possible health effects that made those chemicals selected as a priority by the PITOC project were further supported by new data (as for menthol and cocoa).

The selection of the priority list was based on unfavourable toxicological characteristics of the compounds in their unburnt form or of pyrolysis products, and/or based on possible available information about attractiveness and addictiveness. A data sheet was prepared for each chemical containing the most relevant above mentioned information and includes a paragraph describing the criteria for inclusion into the priority list.

Some chemicals with very similar structures (i.e., aliphatic gamma-lactones) and/or properties (e.g., weak acids) were grouped together. For the aliphatic gamma-lactones, possible criteria for prioritisation within the group were identified on the basis of unfavourable properties, and possible representative additives within the group were identified. For the other 'groups' this was not possible, because there was not enough toxicological information to identify any priority among members of the group that seemed to be of equal concern. In those cases frequency and amount of use were the criteria applied for prioritization.

Some chemicals, included in the initial selection, were excluded from the priority list because data were scant or because the limited available data did not indicate adverse human health effects. However, these chemicals are cited in the Opinion for transparency reasons as "additional chemicals".

For chemicals in the priority list, the information about the toxicological profile is often limited to the oral route of exposure, especially for flavouring substances which are used by food industries or very rarely to the dermal route (when used in the cosmetic production). Additives used in the manufacturing of cigarettes are approved for use in the US by the FDA generally regarded as safe (GRAS) list and/or are indicated as "of no safety concern" by JECFA or EFSA when used in food; in many cases, they are also considered safe by FEMA (Flavour and Extracts Manufacturers Association). However, these evaluations apply to ingredients in foods or cosmetics that are ingested or topically applied, but very rarely relevant for additives in tobacco, which are either transferred to inhaled smoke in pure form, or are combusted and converted into pyrolysis products, which may have toxic effects. Therefore, it is imperative to assess the possible risks of additives in tobacco taking into account that inhalation is the relevant route of exposure.

Data about inhalation toxicity are negligible, as is data on kinetic behaviour that could be eventually used to carry out some route-to-route extrapolation, starting from oral data. Inhalation exposure due to the large surface area in the lungs can have a profound effect on the addictiveness of a toxic product, as well as the inherent toxic potential of the additive; the absence of a real epithelial barrier such as the one on the skin and in the gastrointestinal mucosa usually corresponds to a higher percentage of absorption and consequently a higher internal dose.

For most of the additives, there is scarcity of information on the exposure via the use of tobacco products including exposures resulting from the combustion reactions' products. Data on pyrolysis in the actual condition of use are scant. Limited or no information is available on possible mixture toxicity, also due to lack of knowledge on all the components of the mixture. It must be borne in mind that each of the tobacco additives (and combustion products) is only one component out of the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur, but cannot be adequately evaluated.

For most tobacco additives, direct information about possible effects on addictiveness and attractiveness does not exist (due to the lack of specific testing), but indirect information can be derived based on the mode of action of the single chemical used as additive.

Taking into account these aspects, the list of 30 entries corresponding to chemicals (or groups of chemicals) used as additives in tobacco products has been compiled. It corresponds to a total of 48 single additives listed in Table 2, in which the most important features of the four categories indicated in the terms of reference are reported.

For chemically defined additives, it was possible to identify a unique CAS number, reported in Table 2; SCHENIR is aware that other compounds with similar structure and different CAS number, potentially used as tobacco additives, could be relevant as well. However, since very different toxicological profiles can characterise isomers of the same molecule, it is not possible to generalise. More details about possible grouping will be dealt with in Opinion 2.

The identification with CAS number is an issue for natural compounds and mixtures for which some of the substances' names can be linked to multiple CAS numbers referring to different specific fractions and extracts corresponding to different CAS numbers (Table 1). In general, published papers in the literature do not state the specific CAS number of the tested additive(s) making a clear distinction almost not possible. Considering the similar structures, the effects are expected to be rather the same, although some fraction with different physicochemical properties can possibly affect bioavailability and consequently effects on specific endpoints.

The list of the priority chemicals (in alphabetical order):

1. Acetanisole
2. Aliphatic gamma-lactones (including 8 members: gamma-Valerolactone, gamma-Hexalactone, gamma-Heptalactone, gamma-Octalactone, gamma-Nonalactone, gamma-Decalactone, gamma-Undecalactone, gamma-Dodecalactone)
3. Ammonium Compounds
4. Benzaldehyde

5. Benzoic acid and sodium benzoate (2 members)
6. Benzyl alcohol
7. Caramel colours
8. Carob bean extract
9. Cellulose
10. Cocoa
11. β -Damascone
12. Diacetyl
13. 2-Furfural
14. Geraniol
15. Glycerol
16. Guar gum
17. Guaiacol
18. Linalool
19. Liquorice
20. Maltol
21. Menthol
22. Natural extracts (including 4 members: fenugreek, rum, plum extract, fig extract)
23. Phenylacetic acid
24. Piperonal
25. Propylene glycol
26. Sorbitol
27. Sugars
28. Titanium dioxide
29. Vanillin
30. Weak organic acids (including 8 members : potassium sorbate, sorbic acid, citric acid, acetic acid, butyric acid, lactic acid, 2-methyl butyric acid, potassium citrate)

A list of additional chemicals from the first selection, but later excluded from the priority list includes 8 members:

1. Acetophenone
2. 3,4-dihydrocumarine
3. dimethoxybenzene
4. ethylbutyrate
5. ethylmaltol
6. 4-hydroxy-2,5-dimethyl-3(2H)-furanone
7. ionone
8. 3-methyl cyclopentane-1,2-dione

As explained above, these additional chemicals were excluded from the priority list because data were scant or because the limited available data did not indicate adverse human health effects. This does not mean that they are of no concern.

As indicated in Table 2 and listed below, the compounds in the priority list show one or more of the four characteristics provided for in Article 6.

In summary:

- 17 selected substances fall/are suspected to fall in the category: toxic in unburnt form, among which 6 were suspected of CMR potential.
- 14 selected substances are suspected of facilitating inhalation or increasing nicotine uptake (mechanism possibly contributing to addictiveness to smoking)
- 19 selected substances show a characterising flavour, one of the factors potentially contributing to attractiveness
- 20 selected substances are known or suspected to form irritant, toxic and/or CMR chemicals after combustion.

In the Terms of Reference, it is indicated that the SCENIHR should '*indicate as far as possible 'rankings of additives and provide an explanation for its ranking'*'. In the following, an attempt to give some prioritisation is given, in a few cases based on hazard information (qualitative approach) but in most cases only based on their ranking in frequency and amount used in tobacco products, as listed in the NL list.

For 6 substances, the carcinogenic/genotoxic potential could not be ruled out, with different degree of uncertainty. They are considered as the **first priority** within the priority list, because according to the Tobacco Products Directive 2014/40/EU, Article 7 foresees the prohibition of using additives that have CMR properties in unburnt form.

As it was impossible to quantitatively rank them on the basis of their CMR potential (giving rise to comparable concern), the prioritisation of additives was based on available data concerning their content and frequency of use.

1. Titanium dioxide: classified as Group 2B (possibly carcinogenic to humans)
2. Maltol: genotoxicity concern could not be ruled out
3. Diacetyl: there is uncertainty about the genotoxicity potential. There were no data on carcinogenicity.
4. Geraniol: may contain the relevant impurity methyleugenol, a genotoxic carcinogen
5. Guaiacol: The only genotoxicity test on mammalian cells gave positive results (SCE in human lymphocytes).
6. 2-furfural: showed a co-carcinogenic effect with benzo[a]pyrene, present in cigarette smoke.

The first data requirement for these additives would be to define their CMR potential in their unburnt form. If they tested positive, no further testing would be needed, because that alone would cause them to be banned according to the TPD. If CMR properties in unburnt form were excluded, these compounds would be considered similar to the other additives on the list; indeed, they would fall into additional categories: 3 (Guaiacol), 2 (Geraniol, Diacetyl, Maltol) and 1 (2-furfural and Titanium dioxide) of the four characteristics provided for in Article 6. As for the other additives in the list, the possibility that these compounds give rise to chemicals with CMR properties after combustion should also be verified.

In addition to these 6 chemicals, menthol, one of the most commonly used tobacco additives worldwide, may also be considered high priority, as a wide body of evidence is available to indicate the presence of characterising flavour, as well as a complex

pharmacologic action (including anaesthetic effects, facilitation of deeper inhalation and higher nicotine exposure), characteristics which tobacco additives should not have according to the TPD. However, further data are needed to elucidate the dose-response relationship, to determine whether the described effects are relevant at the actual exposure levels as an additive in “non” menthol brands of tobacco products.

Additives such as sugars and sugar-containing additives (caramel colours, natural extracts, also including carob bean extracts and guar gum) and cellulose are included (ranked on the same basis) in a **second group**, because they are well known to form CMR substances after combustion.

Within the group of sugars, some ‘read across’ techniques can be used for similar molecules, taking care to separate simple sugars from complex structures. Cellulose is clearly different from sugars and it is omnipresent in tobacco products. Natural extracts consist of a cocktail of unknown compounds (beside sugars) with significant differences in their composition: this implies a high level of uncertainty on their potential health effects. Therefore, apart from effects related to CMR substances formed after sugar combustion, it is probably not acceptable to identify a single representative for the group.

All the remaining identified additives are categorised in the **third group**. It was not possible to rank them on the basis of their specific hazard profile. The possible criteria used for their prioritisation are: listing them with respect to their content/frequency ranking or possible combination of more than one of four characteristics provided for in Article 6.

Within the third group, among the structurally-similar compounds benzaldehyde, benzoic acid and sodium benzoate, which during pyrolysis give rise to benzoic acid and sodium benzoate can be considered as a representative compound.

Weak organic acids are supposed to result in relative alike exposures following smoking exposure and therefore can be considered as a group, taking the one with the highest content/frequency as the representative additive.

The aliphatic gamma-lactones (eight molecules in total) represent a set of similar but different compounds. Given the difference between long and short chain lactones, it is suggested to consider two representative lactones within the group with short and long chain length. Another criterion for prioritisation among the group could be related to the content/frequency of use, according to which gamma-hepta-lactone is used at the highest levels although less frequently than others.

The ammonium compounds have a potential as pharmacological active compounds, but given their low content/frequency of use they were ranked lower in the group. Humectants include additives (propylene glycol, sorbitol, glycerol) the content/frequency of which is quite substantial (propylene glycol is ranked 3rd in the NL list and glycerol as 8th).

As a consequence, following these qualitative criteria, the list of priority chemicals may be re-organised as follows:

First group (CMR properties in the unburnt form, sorted by content/frequency plus menthol, characterised by different pharmacological effects):

- Titanium dioxide
- Maltol
- Diacetyl
- Geraniol
- Guaiacol
- 2-furfural
- Menthol

Second group (giving rise to chemicals with CMR properties after combustion sorted by their content/frequency):

- Cellulose
- Sugars
- Caramel colours
- Natural extracts in decreasing ranking according to the NL list: guar gum, rum, fig extract, fenugreek, plum extract, carob bean extracts)

Third group (the remaining additives in the priority list sorted by their content/frequency; additives falling in more than one category (in bold) were placed on top of the group)

- **Cocoa**
- **Piperonal**
- **Benzaldehyde/benzoic acid/sodium benzoate** (Benzoic acid as representative)
- **Benzyl alcohol**
- **Liquorice**
- **Phenylacetic acid**
- **Linalool**
- Humectants (Propylene glycol, Sorbitol, Glycerol; Propylene glycol can be taken as the representative one)
- Vanillin
- Aliphatic gamma-lactones (short chain length: octa-lactone; long chain length: undeca-lactone)
- Weak organic acids (citrate can be used as representative compound)
- Acetanisole
- β -Damascone
- Ammonium compounds

Final remarks and concerns:

Two issues were discussed concerning the proposed list:

Firstly, it is noted that for some substances in the list several structural analogue substances exist (with similar chemical and physical characteristics). Care must be taken if substances on the list are substituted by similar substances that may (or may not) have similar or even higher potential hazardous characteristics, e.g., for the substances 2-furfural, in the first group of the list, several potential analogues exist (e.g., 3-furfural, 5-methyl-furfural, furfuryl alcohol, methyl furfural, etc.). The latter compounds are not the subject of the current Opinion because they did not rank high in use or frequency or are not used at the moment in tobacco products. The SCENIHR stresses that potential substitutes, when not in the current list, are not to be seen as safe.

The second concern of the SCENIHR is related to the diverse nature of natural compounds. Substances reported in the current priority list are derived natural products, which means that different forms exist depending on specific extraction process and fractions, each one corresponding to different CAS numbers. Several examples:

- Cocoa is marketed as cocoa extract, cocoa powder, cocoa butter, cocoa shells, cocoa distillate, cocoa hulls, etc. All these products are similar but also somewhat different since they represent different techniques to collect the natural product.
- Some natural flavours are identified as different substances or forms. E.g. l-menthol, D-menthol, D/L-menthol, cornmint oil, menthone, L-menthyl acetate, peppermint absolute. These flavours all can be linked to menthol but differ significantly in chemical structure and/or composition.
- Vanilla bean extract (a natural extract) and vanillin and ethyl vanillin (synthetic vanilla flavours) are all used as vanilla but differ in chemical structure and/or composition.

In most cases, it is not clear to what extent the toxicological profile of the different forms of natural compounds can be compared, partly due to the fact that published papers in the open literature do not state the specific CAS number of the tested additive(s).

1 **Table 2 – Tobacco additives of concern and their main features regarding toxicity, combustion products, addictiveness and**
 2 **attractiveness**

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects					
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness	
					toxic per se	toxic after combustion	CMR properties			
Acetanisole	220 (0)	100-06-1	0.0057	88	Moderate skin irritant, somnolence (general depressed activity), irritability, and muscle weakness	Unclear				Fragrance 0.003-0.12 weight %.
Aliphatic lactons in general					Very low acute oral toxicity	Data available on 1 of the group (valerolactone) : converted into aromatic hydrocarbons through catalytic pyrolysis	Not expected to be genotoxic	Mild to weak inhibitors of CYP2A6, may increase the addictive effect of nicotine	Important flavour and aroma constituents	
gamma-Valerolactone	266 (0)	108-29-2	0.0017	71		Converted into aromatic hydrocarbons through catalytic pyrolysis,		Mild to weak inhibitors of CYP2A6 may increase the addictive effect of nicotine		
gamma-Hexalactone	180 (0)	695-06-7	0.0029	117				Mild to weak inhibitors of CYP2A6, may increase the addictive effect of nicotine		

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
gamma-Heptalactone	164 (84)	105-21-5	0.2528	133				Mild to weak inhibitors of CYP2A6, may increase the addictive effect of nicotine	Added to tobacco cigarettes for its flavouring properties in amounts of 0.001 % w/w
gamma-Octalactone	375 (0)	104-50-7	0.0017	37					
gamma-Nonalactone	407 (0)	104-61-0	0.0177	31					
gamma-Decalactone	204 (0)	706-14-9	0.0025	96				Mild to weak inhibitors of CYP2A6 may increase the addictive effect of nicotine	
gamma-Undecalactone	269 (0)	104-67-6	0.009	68					Odour Detection Threshold (in other) = 950 ppb
gamma-Dodecalactone	41 (0)	2305-05-7	0.0004	369				Mild to weak inhibitors of CYP2A6 may increase the addictive effect of nicotine	Odour Detection Threshold (in water) = 7 ppb

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Ammonium Compounds (Ammonium Phosphate)	13(5)	100-52-7	0.0679	646				Hypothesised to facilitate inhalation by increasing pH	
Benzaldehyde	519 (0)	100-52-7	0.0165	24	Irritant to skin, eyes and airways, may cause contact dermatitis. CNS depression in low doses, feeble local anaesthetic, classified as a hazardous substance by the U.S. EPA	At 200°C a significant amount (~26%) oxidises to benzoic acid further transformed in CMR compounds			Fragrance 0.001-0.08 weight %
Benzoic acid and sodium benzoate	248 (0)	65-85-0 532-32-1	0.0297	79	Mild skin irritant, mild irritant to the eyes and mucus membranes	Pyrolysis products benzene, phenol, styrene were identified			
Benzyl alcohol	458 (2)	100-51-6	0.1688 Maximum level of use 0.025% w/w as flavour, solvent. (RJRT)	27	Local anaesthetic, irritant to eyes, nose and throat, skin sensitiser, may cause drowsiness, dizziness, fragrance allergen, considered harmful by inhalation			Local anaesthetic, facilitates inhalation	Odour threshold 5.5 ppm
Caramel colours	288 (183)	8028-89-5	0.0535 Maximum use 0.00003 % (BAT German domestic) Max Level of Use in Any Cigarette Brand 0.01% w/w	58		Pyrolysis of sugars leads to formation of a number of CMR substances			Characterising flavour

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Carrob bean extract	59(0)	9000-40-2 84961-45-5	0.0054	298		Pyrolysis of sugars leads to formation of a number of CMR substances			
Cellulose	1376(1043)	9004-34-6 (various, e.g. 65996-61-4 232-674-9, (carboxymethyl cellulose: 900-11-7)	2.1641	5		Pyrolysis of cellulose leads to formation of a number of CMR substances			
Cocoa	847(0)	84649-99-0 (cocoa extract) 95009-22-6 (cocoa powder) 84649-99-3 (cocoa extract) 8002-31-1	0.1053	14		Pyrolysis of sugars leads to formation of a number of CMR substances		Facilitates inhalation through the action of bronchodilator of some metabolites, as well with inhibition of MAO activities by other metabolites	Enhances smoke flavour
β-damascone	210 (0)	23726-92-3 35044-68-9 23726-91-2	0.000076	92		pyrolysis products benzene, toluene, anthracene and phenanthrene were identified			

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Diacetyl	296 (0)	431-03-8	0.0038	53	Diacetyl exposure may lead to lung disease after inhalation		SCOEL accepted that there is uncertainty about the importance of the genotoxicity of diacetyl. There were no data on carcinogenicity .		It imparts a buttery sweet taste and a buttery odour, one of the characterising flavours of caramelised foods.
2-furfural	71 (0)	98-01-1 (4;6;7)	0.0083	262			2-furfural showed a co-carcinogenic effect with benzo[a]pyrene, present in cigarette smoke.		It has a sweet caramel-like flavour
Geraniol	267 (2)	106-24-1	0.0015	69	Irritant, geraniol and geraniol oxidation products (i.e. geraniol, epoxy-geraniol, epoxy-geraniol) are sensitisers		Can contain the relevant impurity methyleugenol , a genotoxic carcinogen		Resulting in a characterising flavour: Odour Detection Threshold (in water) = 40-75 ppb

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Glycerol	1000(37)	56-81-5	0.793	8		Acrolein is a toxic pyrolysis product of glycerol, which is highly reactive and causes irritation in the respiratory tract.		Humectant, reduces the harshness of cigarette smoke, facilitates inhalation	
Guar gum	937 (620)	9000-30-0 68411-94-9 (Guar depolymerised)	0.243	10		Pyrolysis of sugars leads to formation of a number of CMR substances. When heated, emits acrid smoke and irritating fumes			
Guaiacol	219 (0)	90-05-1	0.0015	89	Severe eye irritant, as skin irritant and it is also reported to be a respiratory tract irritant (generating hyperplasia)		The only genotoxicity test on mammalian cells gave positive results (SCE in human lymphocytes)	Local anaesthetic can favour the smoke inhalation	Resulting in a characterising flavour: Odour detection threshold: 3-21ppb.
Linalool	277 (2)	78-70-6	0.0043 used as a flavouring, maximum use of 0.00001 % w/w (BAT	62	Classified as skin sensitiser 1A- H317			Local anaesthetic can favour the smoke inhalation	Resulting in a characterising flavour: Odour detection threshold: 6ppb.

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Liquorice	402 (0)	68916-91-6 84775-66-6	0.1541	34		Pyrolysis of sugars leads to formation of a number of CMR substances			Enhance smoke flavour
Maltol	534 (0)	118-71-8	0.0025	23	Possible effect on the CNS has to be clarified (inhibition of the response of GABA _A receptors)		Genotoxicity concern could not be ruled out		Reported as flavour, conc: 220 ppm
Menthol	139 (45)	l-Menthol: 2216-51-5 D-Menthol: 15356-70-4 D/L Menthol: 89-78-1 Menthol: 1490-04-6	0.3928	154				Local anaesthetic, facilitates inhalation, enhance nicotine bioavailability, possibly affects addictiveness due to higher nicotine uptake	Increases attractiveness of smoking, especially for young people

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Natural extracts in general					Generally recognised as safe as food additives and flavours	Upon combustion/pyrolysis at temperatures (up to 900°C) attained during smoking, these compounds, especially the carbohydrates will give rise to a complex mixture of toxic, carcinogenic, mutagenic compounds, besides aroma/flavour compounds. Compounds formed include soothing agents (e.g. organic acids), flavours (e.g. caramel), facilitating nicotine delivery (e.g. aldehydes) and with CMR properties (e.g. PAHs, formaldehyde). For more details see plum extract, fenugreek, sugars and cellulose.		Compounds formed include soothing agents (e.g. organic acids), facilitating nicotine delivery (e.g. aldehydes)	Give characterising flavour

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Fenugreek	206 (0)	84625-40-1 68990-15-8, 8023-90-3, 977018-53-3, (extract, resin, absolute)	0.0126 maximum use 0.00451% w/w as flavouring (BAT-Germany) Fenugreek oleoresin, max. use 0.00068% w/w as flavouring (BAT-Germany)	95	Fenugreek is "generally recognised as safe" (GRAS) as a flavouring by the US Food and Drug Administration.	Pyrolysis of sugars leads to formation of a number of CMR substances		Compounds formed include soothing agents (e.g. organic acids), facilitating nicotine delivery (e.g. aldehydes)	Give characterising flavour
Rum	281 (0)	91450-09-8 90604-30-1	0.1046 Rum or rum extract used as flavour at the level QNE 0.01% by JTI Germany	60				Compounds formed include soothing agents (e.g. organic acids), facilitating nicotine delivery (e.g. aldehydes)	Rum or rum extract used as flavour at the level QNE 0.01% by JTI Germany
Fig extract	229 (0)	90028-74-3	0.0889 Fig Juice Concentrate used at <0.0001 % as flavour (RJRT); Fig juice concentrate: maximum level of use 0.00220 % w/w as flavouring (BAT).	84		Combustion products from the high sugar/ carbohydrate concentration would include aldehydes (can potentiate effect of nicotine), flavour compounds (increase palatability) and toxic carcinogenic compounds		Compounds formed include soothing agents (e.g. organic acids), facilitating nicotine delivery (e.g. aldehydes)	Fig Juice Concentrate used at <0.0001 % as flavour (RJRT); Fig juice concentrate: maximum level of use 0.00220 % w/w as flavouring (BAT).

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Plum extract	9 (0)	90082-87-4 Prune Concentrate: 83173-17-5	0.0476	733		Pyrolysis of sugars leads to formation of a number of CMR substances			
Phenylacetic acid	331 (0)	103-82-2	0.0032 0.00038 % w/w (BAT German domestic), 0.0001 % w/w in tobacco as flavouring	46	A potential respiratory irritant, it can cause damage to the respiratory tract following inhalation. Acute exposure can cause redness of skin and redness and pain in the eyes; inhalation can cause cough/sore throat;	Decomposes upon burning, produces irritating fumes, no info on combustion products			Floral odour, Odour Threshold: 10000 ppb
Piperonal	591 (0)	120-57-0	0.0314	20	Irritant, sensitiser, possible CNS effect			Might elevate mood and well-being, some studies indicating that piperonal has psycho-active effects, such as anxiety reduction	fragrance 0.003-03 weight %
Propylene glycol	1599(23)	57-55-6	1.5789	3		Formation of possibly toxic (CMR) compound after combustion		Humectant, reduces the harshness of cigarette smoke, facilitates inhalation	

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Sorbitol	210 (30)	50-70-4	0.458	93		Pyrolysis compounds: 2-furfural (31.4 % see section on furfural), acetaldehyde (irritant and possible human carcinogen), formaldehyde (irritant, carcinogen).		Humectant, reduces the harshness of cigarette smoke, facilitates inhalation	
Sugars	767 (0)	50-99-7 (glucose) 977042-84-4 (High fructose corn syrup) 68476-78-8, 8052-35-5 Molasses, sugar cane)	2.734	17		Pyrolysis of sugars leads to formation of a number of CMR substances			
Titanium dioxide	1329 (1256)	13463-67-7 mixture of mainly rutile and anatase 1317-80-2 rutile 1317-70-0 anatase	0.161	6	Inhalation toxicity (acute inflammation in lungs after repeat dose) Because inhalation toxicity is also related to the size of the particles, a distinction needs to be made between nano- and non-nano size.		Classified as Group 2B (possibly carcinogenic to humans)		

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Vanillin	860(0)	121-33-5	0.1647	12		Thermal decomposition or burning may release carbon monoxide or other hazardous gases, acrid smoke and irritating fumes			Characterising flavour
Weak organic acids in general					According to available data, weak organic acids do not present a hazard for the human health based on its low hazard profile	After combustion mostly in the intact form		They are suspected to ease smoking, and facilitate the inhalation and absorption of nicotine, potentially resulting in increased addictiveness, although no direct evidence exists in this respect	As flavouring compounds may increase attractiveness
Potassium sorbate	242 (54)	590-00-1 24634-61-5	0.0598	81	Eye irritation	Completely pyrolysed to form aromatic ring materials including benzene, toluene, substituted benzenes, naphthalene, and substituted naphthalenes			

Additives used in tobacco products

Compound	frequency of use (NTM) - NL list	CAS number	Average of ingredient % w/w (NL list)	Ranking NL list	Human health effects				
					Toxicity			Addictiveness/ facilitating inhalation	Attractiveness
					toxic per se	toxic after combustion	CMR properties		
Sorbic acid	271 (19)	110-44-1	0.0198	65		Pyrolysis products – phenol (0.5 % - 0.0003 µg) and decanal (0.1% - 0.0005 µg)			
Citric acid	323 (55)	77-92-9	0.0737	47					
Acetic acid	290 (8)	64-19-7	0.0417	55	Irritant				
4-Butyric acid	288 (0)	107-92-6	0.007	57					
Lactic acid	248 (0)	50-21-5 79-33-4 598-82-3	0.0477	78					
2-methyl butyric acid	194 (0)	116-53-0	0.0006	109					
Potassium citrate	593 (549)	866-84-2; 6100-05-6	0.1063	19	EU-permitted Food Additive (E 332);causes mild irritation to respiratory tract, to skin, and eyes				

5 MINORITY OPINION

None.

6 CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS

A public consultation on this Opinion was opened on the website of the non-food scientific committees from 22 July 2015 to 23 September 2015. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

Thirty-seven different contributors (providing in total 266 comments) participated in the public consultation providing input to different parts of the Opinion. Majority of comments came from the tobacco industry, several came from public health authorities/institutes. Thirty-five comments were marked as confidential. The SCENIHR provided an individual reply to each contributor.

Each submission was carefully considered by the SCENIHR and the scientific Opinion has been revised to take account of relevant comments. The literature has been accordingly updated with relevant publications. However, in the Final Opinion, the priority list was unchanged, although editorial changes were made to address comments pointing out possible misunderstanding.

The most frequent comments were related to methodological aspects (e.g. literature search, choice of the representative list, lack of conducting a thorough risk assessment, etc.). Tobacco Industry repeatedly criticised the Preliminary Opinion supposed to go beyond the Terms of References when examining properties such as 'attractiveness' or 'addictiveness', and expressed complaints about the identification of data gaps. They also asked to distinguish between blended cigarettes, which use additives, and Virginia cigarettes, which use no or very few tobacco additives and to consider factors such as 'societal influences' in smoke addiction. However, these latter comments and those related to risk management measures were considered to be outside of the SCENIHR mandate, which relates to risk assessment.

To this aim the methodological paragraph was completely re-written in order to give more details on the literature search, to adapt the Weight of Evidence at the specific approach used, to explain the choice of the typical list, the use of information coming from the PITOC project and the reasons for not conducting an exhaustive toxicological evaluation. Issues regarding 'attractiveness' or 'addictiveness' were specified and clarified. The choice of the list from the Netherlands was explained in more details.

The explanation for not conducting an exhaustive toxicological evaluation and related risk assessment was changed, removing 'time constraints' – a wording that was intentionally misinterpreted: 'this method enabled identifying a number of priority substances based on hazard identification; therefore, a full risk assessment of the large number of compounds was not carried out.'

The many papers provided by the Tobacco industry were checked, but in most cases were considered as not providing any additional information or any information relevant enough to change the Opinion.

For example, data on specific mixtures cannot be used to make general conclusions. And the additives selection was based on properties of additives *per se* and not in conjunction with the tobacco matrix, which has been established as toxic.

The text of the comments received and the response provided by the Scientific Committees is available here:

http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_29_en.htm

7 ABBREVIATIONS AND GLOSSARY OF TERMS

BAT	British American Tobacco
CAS	Chemical Abstracts Service
CNS	Central nervous system
CO	Carbon monoxide
CYP	Cytochrome P450 monooxygenase
DKFZ	Deutsches Krebsforschungszentrum (German Cancer Research Centre)
EC	European Commission
ECDC	European Centre for Disease prevention and Control
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EU	European Union
FEMA	(US) Flavor and Extract Manufacturers Association
FDA	(US) Food and Drug Administration
GABA	Gamma (γ)-Aminobutyric acid
GRAS	generally recognized as safe
IARC	International Agency for Research on Cancer
IC50	The half-maximal inhibitory concentration
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JTI	Japan Tobacco Inc.
MoE	Mode of Exposure
MRI	Magnetic resonance imaging
NOAEL	No observed adverse effect level
NTM	Non-tobacco material
NTP	US National Toxicology Programme
OECD	Organisation for Economic Co-operation and Development
pH	Measure of acidity or basicity of a solution
PITOC	EU project "Public Information Tobacco Control"
PMI	Philip Morris International
ppm	parts per million
QNE	Quantity not exceeded
RfD	Reference dose
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (The Netherlands National Institute for Public Health and the Environment)
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety

SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SCOEL	Scientific Committee on Occupational Exposure Limits
STEL	Short-term exposure limit
UK	United Kingdom
US(A)	United States (of America)
WHO	World Health Organization
WoE	Weight of evidence

8 REFERENCES

- Adam, T., Mitschke, S., Streibel, T., Baker, R.R., Zimmermann, R. (2006). Puff-by-puff resolved characterisation of cigarette mainstream smoke by single photon ionisation (SPI)-time-of-flight mass spectrometry (TOFMS): comparison of the 2R4F research cigarette and pure Burley, Virginia, Oriental and Maryland tobacco cigarettes. *Anal Chim Acta*, 572, 219-229.
- Aguedo, M., Beney, L., Waché, Y., Belin, J.M. (2003) Interaction of an odorant lactone with model phospholipid bilayers and its strong fluidizing action in yeast membrane. *Int J Food Microbiol.*80(3), 211-5.
- Alpert, H.R., Agaku, I.T., Connolly, G.N. (2015). A study of pyrazines in cigarettes and how additives might be used to enhance tobacco addiction. *Tob Control* 2015Jun 10. doi: 10.1136/tobaccocontrol-2014-051943.
- Arnarp J., Bielawski, J., Dahlin, B.M., Dahlman, O., Enzell, CR., Pettersson, T. (1989). Tobacco smoke chemistry. 2. Alkyl and alkenyl substituted guaiacols found in cigarette smoke condensate. *Acta Chem Scand.* 43(1), 44-50.
- Asakawa, E., Hirose, M., Hagiwara, A., Takahashi, S., Ito, N. (1994). Carcinogenicity of 4-methoxyphenol and 4-methylcatechol in F344 rats. *Int. J. Cancer* 56(1), 146-152.
- Baker, RR, Massey, ED, Smith, G. (2004). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food Chem Toxicol.*, 42S, 53-83.
- Baker R.R, Pereira da Silva J.R, Smith G (2004). The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives. *Food and Chem. Toxicol.*42S: S39-S52.
- Baker R.R, Pereira da Silva J.R, Smith G (2004). The effect of tobacco ingredients on smoke chemistry. Part II: Casing ingredients *Food and Chem. Toxicol.* 42S: S3-S37
- Baker, R.R., Bishop, L.J. (2004). The pyrolysis of tobacco ingredients. *J. Anal. Appl. Pyrolysis* 71, 223-311.
- Baker, R.R., Bishop, L.J. (2005). The pyrolysis of non-volatile tobacco ingredients using a system that simulates cigarette combustion conditions. *J Anal Appl Pyrolysis.* 74, 145-170.
- Ballantyne, M. (2012). Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. Maltol. Covance Laboratories LTD. Study no. 8250465. January 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Bar, V.F., Griepentrog, F., (1967). Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. *Medizin Ernähr* 8, 244-251.
- Bates, C., Jarvis, M., Connolly, G. (1999). Tobacco Additives: cigarette engineering and nicotine addiction. London: ASH UK Report.

Beevers, C. (2013). Combined bone marrow micronucleus test and comet assay in the liver of treated rats. Maltol. Covance Laboratories Ltd. Study no. 8262049. February 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., (2007). A toxicologic and dermatologic assessment of ionones when used as fragrance ingredients. Food and Chemical Toxicology 45, 130–167.

BIBRA working group; TA: Toxicity profile. BIBRA Toxicology International: 4 (1991).

Bjeldanes, L.F., Chew, H. (1979). Mutagenicity of 1,2-dicarbonyl compounds: Maltol, kojic acid, diacetyl and related substances. Mutat Res Toxicol. 67(4), 367–371.

British American Tobacco, The facts about tobacco ingredients, 2014 <http://www.bat-ingredients.com/>.

Born, S.L., Rodriguez, P.A., Eddy, C., Lehman – McKeeman, L.D. (1997). Synthesis and reactivity of coumarin 3,4 - epoxide. Drug Metabolism and Disposition 25, 1318 – 1323.

Buchbauer, G., Jirovetz, L., Jager, W., Plank, C., Dietrich, H. (1993). Fragrance compounds and essential oils with sedative effects upon inhalation. J.Pharm.Sci. 82(6), 660-664.

Burleighflayer, H., Dodd, D., Frank, F., Calisti, L., Walker, J., Nabisco,R. (1990). Evaluation Of Sensory Irritation Potential And Assessment Of The Respiratory Response During Exposure To Acetic Acid Vapor. Bushy Run Research Center, Union Carbide.

Cahours X., Verro Th., and Steve Purkis. (2012). Effect of Sugar Content on Acetaldehyde Yield in Cigarette Smoke. Contributions to Tobacco Research Volume 25 No. 2; 381-395.

Carmines, E.L. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1: cigarette design, testing approach, and review of results. Food Chem Toxicol. 40(1), 77-91.

Chemical Safety Information from Intergovernmental Organizations (INCHEM), IPCS International Programme on Chemical Safety, Benzyl Alcohol <http://www.inchem.org/documents/icsc/icsc/eics0833.htm>

Chemical Safety Information from Intergovernmental Organizations (INCHEM), Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives 2-Methylbutyric Acid, 2001 http://www.inchem.org/documents/jecfa/jeceval/jec_1436.htm

Chemical Safety Information from Intergovernmental Organizations (INCHEM), Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives Citric Acid, 2001. http://www.inchem.org/documents/jecfa/jeceval/jec_436.htm

Chemical Safety Information from Intergovernmental Organizations (INCHEM), Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives Phenylacetic Acid, 2003. http://www.inchem.org/documents/jecfa/jeceval/jec_1886.htm

Chemical Safety Information from Intergovernmental Organizations (INCHEM), Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives

Potassium Dihydrogen Citrate, 2003.
http://www.inchem.org/documents/jecfa/jeceval/jec_1973.htm

Chemical Safety Information from Intergovernmental Organizations (INCHEM), Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives Acetanisole, 2003. http://www.inchem.org/documents/jecfa/jeceval/jec_9.htm

Chemical Safety Information from Intergovernmental Organizations (INCHEM), Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives Lactic Acid, 2005 http://www.inchem.org/documents/jecfa/jeceval/jec_1252.htm

Chemical Safety Information from Intergovernmental Organizations (INCHEM), Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives Sorbic Acid, 2005. http://www.inchem.org/documents/jecfa/jeceval/jec_2181.htm

Coggins, C.R.E., Sena, E.J., Langston, T.B., Oldham M.J. (2011). A comprehensive evaluation of the toxicology of cigarette ingredients: aromatic carbonyl compounds. *Inhalation Toxicology* 23, 90-101.

Coggins, C.R.E., Jianmin, L., Merski, J.A., Werley, M.S., Oldham, M.J. (2011). A comprehensive evaluation of the toxicology of cigarette ingredients: aliphatic and aromatic carboxylic acids *Inhalation Toxicology*. 23, 119–140.

Cosmetic Ingredient Review Expert Panel (2003). *International Journal of Toxicology* 22(suppl. 2), 1-10.

Enomoto, M., (1987).Safrole. In: Hirono, I. (Ed.), *Bioactive Molecules, Vol. II, Naturally Occurring Carcinogens of Plant Origin*. Elsevier, NewYork, pp. 139–159.

European Chemical Agency (ECHA) (2014) CLH report Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2 Substance Name: Linalool available at: <http://echa.europa.eu/documents/10162/51b5de87-ca6d-45f1-9c46-4717698bd049>

European Food Safety Authority (EFSA) Panel on Food additives, flavourings, processing aids and materials in contact with food (AFC) (2004).Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on Titanium dioxide. *EFSA Journal* 163, 1-12.

European Food Safety Authority (EFSA) Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) (2008).Flavouring Group Evaluation 58 (FGE.58). Consideration of phenol derivatives evaluated by JECFA (55th meeting) structurally related to ring substituted phenolic substances evaluated by EFSA in FGE.22 (2006). *EFSA Journal* 711, 1-50.

European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (2008). Flavouring Group Evaluation 80, Revision 1 (FGE.80Rev1): Consideration of alicyclic, alicyclic-fused and aromatic-fused ring lactones evaluated by JECFA (61st meeting) structurally related to a aromatic lactone evaluated by EFSA in FGE.27 (2008). *EFSA Journal* 1169, 1-32.

European Food Safety Authority (EFSA). (2008). Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request

from Commission on Flavouring Group Evaluation 69, (FGE.69) Aromatic substituted secondary alcohols, ketones and related esters. The EFSA Journal (2008) 869, 1-35.

European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (2011). Scientific Opinion on Flavouring Group Evaluation 22, Revision 1 (FGE.22Rev1): Ring-substituted phenolic substances from chemical groups 21 and 25. EFSA Journal 9(5), 1990.

European Food Safety Authority (EFSA) (2011). Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 218, Revision 1 (FGE.218Rev1): alpha,beta-Unsaturated aldehydes and precursors from subgroup 4.2 of FGE.19: Furfural derivatives. EFSA Journal 9, 1840.

European Food Safety Authority (EFSA) (2012). Refined exposure assessment for caramel colours (E 150a, c, d)¹, EFSA Journal, 10(12), 3030.

European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (2012). Scientific Opinion on the safety and efficacy of alicyclic and aromatic lactones (chemical group 11) when used as flavourings for all animal species. EFSA Journal 10(3), 2622.

European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). (2012). Scientific Opinion on the safety and efficacy of benzyl alcohols, aldehydes, acids, esters and acetals (chemical group 23) when used as flavourings for all animal species. EFSA Journal, 10(7), 2785.

European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP).(2012). Scientific Opinion on the safety and efficacy of aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers (chemical group 6) when used as flavourings for all animal species.EFSA Journal, 10(11), 2966.

European Food Safety Authority (EFSA).(2012). Conclusion on the peer review of the pesticide risk assessment of the active substance geraniol.EFSA Journal 10(11), 2915.

European Food Safety Authority EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (2012). Scientific Opinion on Flavouring Group Evaluation 10, Revision 3 (FGE.10Rev3): Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30. EFSA Journal 2012; 10(3), 2563.

European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (2014a). Scientific Opinion on Flavouring Group Evaluation 213, Revision 1 (FGE.213Rev1): Consideration of genotoxic potential for α,β -Unsaturated Alicyclic ketones and precursors from chemical subgroup 2.7 of FGE.19. EFSA Journal;12(5), 3661.

European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (2014b) Scientific Opinion on Flavouring Group Evaluation 210, Revision 1 (FGE.210Rev1): Consideration of genotoxic potential for α,β -

unsaturated alicyclic ketones and precursors from chemical subgroup 2.4 of FGE.191 EFSA Journal 2014;12(2), 3587.

European Food Safety Authority EFSA (2015). Scientific Opinion on Flavouring Group Evaluation 220 Revision 3 (FGE.220Rev3): Consideration of genotoxic potential for α,β -unsaturated 3(2H)-Furanones from subgroup 4.4 of FGE.19 1 EFSA Journal 2015, 13(5), 4117.

FAO/WHO Expert Committee on Food Additives (1970)., Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives, FAO Nutrition Meetings Report Series No. 48A WHO/FOOD ADD/70.39. <http://www.inchem.org/documents/jecfa/jecmono/v48aje07.htm>

FCTXAV Food and Cosmetics Toxicology. London, UK (1974). V.1-19, 1963-81.

FDA Tobacco Products Scientific Advisory Committee (TPSAC) (2011).Menthol Cigarettes and Public Health: Review of the Scientific Evidence and Recommendations. <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/TobaccoProductsScientificAdvisoryCommittee/UCM247689.pdf>

FDA (2013). Preliminary scientific evaluation of the possible public health effects of menthol versus nonmenthol cigarettes. Available at <http://www.fda.gov/downloads/ScienceResearch/.../UCM361598.pdf>

Feuer, G., Goldberg, L., Gibson, K.I. (1966). Liver response tests.VII Coumarin metabolism in relation to inhibition of rat - liver glucose - 6 - phosphatase. Food and Cosmetics Toxicology, 4, 157 - 167.

Fowles, J. (2001). Chemical factors influencing the addictiveness and attractiveness of cigarettes in New Zealand.New Zealand Ministry of Health.

Fujioka K, Shibamoto T. (2006). Determination of toxic carbonyl compounds in cigarette smoke. Environ Toxicol. 21(1), 47-54.

Gaworski, CL., Lemus-Olalde, R., Carmines, EL. (2008). Toxicological evaluation of potassium sorbate added to cigarette tobacco. Food Chem Toxicol.146(1), 339–351.

Gosselin, R.E., Hodge, H.C., Smith, R.P., Gleason, M.N. (1976). Clinical toxicology of commercial products. 4th ed. Baltimore: Williams and Wilkins.

Green, J.D., Chalmers, J., Kinnard, P.J. (1989). The transfer of tobacco additives to cigarette smoke: Examination of the possible contribution of pyrolysis products to mainstream smoke composition. Beitrage zur TabakforschungInternational, 14, 283-288.

GUIDECHEM Chemical trading guide Fenugreek CAS No. 68990-15-8 <http://www.guidedchem.com/reference/dic-420164.html>

Ha, M.A., Smith, G.J., Cichocki, J.A., Fan, L., Liu, Y.S., Caceres, A.I., Jordt, S.E., Morris, J.B. (2015). Menthol attenuates respiratory irritation and elevates blood cotinine in cigarette smoke exposed mice. PLoS One. 2015 Feb 13;10(2):e0117128. doi: 10.1371/journal.pone.0117128. PMID: 25679525.

Haddouk, H. (2001). Bacterial reverse mutation test. ST05C01. CIT, Evreux, France. Study no. 21664 MMJ. 11 July 2001. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Hall, JB., Sprecker, MA., Shuster, EJ., Schmitt, FL., Vinals, JF. (1979) United States Patent: 4155867 - Substituted dimethyl dihydroxy benzene and cyclohexadiene compounds and uses thereof for augmenting or enhancing the taste and/or aroma of consumable materials including tobaccos, perfumes and perfumed articles [Internet]. 4155867, [cited 2015 Mar 20]. Available from: <http://patft.uspto.gov/netacgi/nph-Parser?d=PALL&p=1&u=%2Fnethtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=4155867.PN.&OS=PN/4155867&RS=PN/4155867>

Hossain, S.J., Aoshima, H., Koda, H., Kiso, Y., (2003) Effects of Coffee Components on the Response of GABAA Receptors Expressed in Xenopus Oocytes. *J Agric Food Chem.* 51(26), 7568–75.

Hurt, R.D., Robertson, C.R. (1998). Prying open the door to the tobacco industry's secrets about nicotine: The Minnesota Tobacco Trial. *JAMA* 280, 1173-81.

<http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3367>

<http://industrydocuments.library.ucsf.edu/tobacco/docs/trdd0100>

<http://www.inchem.org/documents/jecfa/jecmono/40abcj37.htm>

http://www.china-musashino.com/pages/news4_en.htm<https://www.rjrt.com/tobaccoingredients.aspx>

<http://www.health.govt.nz/system/files/documents/pages/tobacco-returns-2013-bat.pdf>

http://www.pmi.com/eng/our_products/pages/technical_products_information.aspx

<http://webnet.oecd.org/HPV/UI/handler.axd?id=fd79fce6-c7e2-48ed-aead-8728c961980c>

IARC (2010). Carbon Black, Titanium Dioxide, and Talc. IARC Monographs on the Evaluation 19 of Carcinogenic Risks to Humans 93, 193-214.

International Toxicity Estimates for Risk (ITER). https://iter.ctc.com/publicURL/p_report_view_list.cfm?crn=98%2D86%2D2

Jansson, T., Curval, M., Hedin, A., Enzell, C.R. (1986). In vitro studies of biological effects of cigarette smoke condensate: II. Induction of sister-chromatid exchanges in human lymphocytes by weakly acidic, semivolatile constituents. *Mutat Res Toxicol.* 1986 169(3), 129–139.

Jansson, T., Curvall, M., Hedin, A., Enzell, C.R. (1988). In vitro studies of the biological effects of cigarette smoke condensate. III. Induction of SCE by some phenolic and related constituents derived from cigarette smoke: A study of structure-activity relationships. *Mutat Res Toxicol.* 206(1), 17–24.

Japan chemical industry ecology-toxicology & information center (jetoc), (2005).Japan; Mutagenicity test data of existing chemical substances based on the toxicity investigation system of the industrial safety and health law, supplement 3.

Jenner, PM., Hagan, EC., Taylor, JM., Cook, EL., Fitzhugh, OG. (1964). Food flavorings and compounds of related structure. I. Acute oral toxicity. Food and Cosmetics Toxicology 2, 327-343.

Joint FAO/WHO Expert Committee on Food Additives (JECFA), (1966).Toxicological Evaluation Of Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-Treatment Agents, Acids And Base, FAO Nutrition Meetings Report Series No. 40A,B,C WHO/Food Add./67.29;<http://www.inchem.org/documents/jecfa/jecmono/40abcj37.htm>

Joint FAO/WHO Expert Committee on Food Additives (JECFA), (1968). The Eleventh Report of the Joint FAO/WHO Expert Committee on Food Additives is published as FAO Nutrition Meetings Report Series,1967, No. 44; WHO Rep. Ser. 383. <http://www.inchem.org/documents/jecfa/jecmono/v44aje32.htm>

Joint FAO/WHO Expert Committee on Food Additives (JECFA), (1969). FAO Nutrition Meetings Report Series No. 46A WHO/FOOD ADD/70.36.

Joint FAO/WHO Expert Committee on Food Additives (JECFA), (1974).Toxicological evaluation of some foodadditives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents, WHO Food Additives Series No. 5.<http://www.inchem.org/documents/jecfa/jecmono/v05je18.htm>

Joint FAO/WHO Expert Committee on Food Additives (JECFA), (2004). Evaluation of certain food additives. Sixty first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 922. Rome, 10-19 June 2003.

Joint FAO/WHO Expert Committee on Food Additives (JECFA), (2005). Evaluation of certain food additives. Sixty-third report of the Joint FAO / WHO Expert Committee on Food Additives http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf

Joint FAO/WHO Expert Committee on Food Additives (JECFA), (2006).Evaluation of certain food additives. Sixty-fifth report of the Joint FAO / WHO Expert Committee on Food Additives.

JT International SA, Ingredients added to tobacco in Germany,<http://ingredients.jti.com/added/GERMANY/>

Juvonen, R.O., Gynther, J., Pasanen, M., Alhava, P., Poso, A. (2000).Pronounced differences in inhibition potency of lactone and non-lactone compounds for mouse and human coumarin 7-hydroxylases (CYP2A5 and CYP2A6). Xenobiotica, 30, 81-92.

Kaighen, M., Williams, R.T. (1961).The metabolism of (3-14C) coumarin. Journal of Medicinal Chemistry, 3, 25-43.

Kalianos, A.G. (1976). Phenolics and acids in leaf and their relationship to smoking quality and aroma.Rec. Adv. Tob. Sci. 2, 61-79.

Kibet, J., Khachatryan, L., Dellinger, B.(2012). Molecular products and radicals from pyrolysis of lignin. Environ Sci Technol.4(46), 12994-3001.

Kovats, E., Demole, E., Ohloff, G., Stoll, M. United States Patent: 4187863 - Cycloaliphatic unsaturated ketones as odor and taste modifying agents in tobacco products.

Kreiss K. (2007) Emerging opportunities to prevent occupational lung disease. *Occup Environ Med.* 64(8):499-500.

Laham, S., Broxup, B., Robinet, M., Potvin, M., Schrader, K. (1991). Subacute inhalation toxicity of benzaldehyde in the Sprague-Dawley rat. *Am. Ind. Hyg. Assoc. J.*, 52(12), 503-551.

Lalko, J., Lapczynski, A., Letizia, C.S., Api, A.M. (2007). Fragrance material review on cis- β -damascone. *Food Chem Toxicol.* 45(1, Supplement 1), 192-198.

Lalko J., Lapczynski, A., Letizia, C.S., Api, A.M. (2007a). Fragrance material: review on ionone. *Food and Chemical Toxicology* 45 (2007) S251-S257.

Lewis, R.J. *Sax's Dangerous Properties of Industrial Materials*. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 22.

Lewis, R.J. *Sax's Dangerous Properties of Industrial Materials*. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 2716.

Light, K.K., Spencer, B.M., Vinals, J.F., Kiwala, J., Vock, M.H., Shuster, E.J. (1979). United States Patent: 4135520 - Tobacco flavoring with cyclohexadiene derivatives.

Light, K.K., Spencer, B.M., Vinals, J.F., Kiwala, J., Vock, M.H., Shuster, E.J. (1978). United States Patent: 4126140 - Smoking tobacco and smoking tobacco flavoring compositions containing hydroxy cyclohexenone derivatives.

Liu, C., Parry A. (2003). Potassium Organic Salts as Burn Additives in Cigarettes. *Beiträge zur Tabakforschung International/Contributions to Tobacco Research* 20(5).

Liu, J.R., Sun, X.R., Dong, H.W., Sun, C.H., Sun, W.G., Chen, B.Q., Song, Y.Q., Yang, B.F. (2008). beta-Ionone suppresses mammary carcinogenesis, proliferative activity and induces apoptosis in the mammary gland of the Sprague-Dawley rat. *Int J Cancer.* 122(12), 2689-2698.

Lloyd, M. (2012). Induction of micronuclei in cultured human peripheral blood lymphocytes. alpha-Damascone. Covance Laboratories LTD. Study no. 8233102. July 2012. Unpublished report Submitted by EFFA to FLAVIS Secretariat.

Lloyd, M. (2013). Final summary report. Induction of micronuclei in cultured human peripheral blood lymphocytes. alpha-Damascone. Covance Laboratories LTD. Study no. 8272016. April 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Makaruk, M.I., Vagonova, L.V. (1985). Toxicological characteristics of acetylanisole. *Gig Sanit.* 4, 86-87. 50(4), 86. <http://www.ncbi.nlm.nih.gov/pubmed/4007552?dopt=AbstractPlus>: *Gigiena i Sanitariya*.

Martínez Enriquez, M.E., Del Villar, A., Chauvet, D., Lopez Valle, A., Susano Pompeyo, M., Campos Sepúlveda, A.E. (2009). Acute toxicity of guaiacol administered subcutaneously in the mouse. *Proc West Pharmacol Soc* 52, 92-93.

Miller, C.W., Dickerson, J.P. Rix, C.E. (1975). Patent Tobacco product US 3996941 A.

Monographs on fragrance raw materials. New York: Pergamon Press, 1979 reviewed in The Hazardous Substance Data Bank (HSDB), The National Library of Medicine (NLM) 2004.

Mpountoukas, P., Vantarakis, A., Sivridis, E., Lialiari T. (2008). Cytogenetic study in cultured human lymphocytes treated with three commonly used preservatives. *Food and Chemical Toxicology*, 46, 7, 2390–2393.

Münzner, R., Guigas, C., Renner, H.W. (1990). Re-examination of potassium sorbate and sodium sorbate for possible genotoxic potential. *Food Chem Toxicol.* 28(6), 397–401.

Musashino Chemical (China) Co., Ltd, Applications. Industry http://www.china-musashino.com/pages/news4_en.htm

Nair, B. (2001a) Final report on the safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. *Int J Toxicol.* 20, 23-50.

Nair, U. (2012). Report. Additives in Tobacco Products. https://www.dkfz.de/de/tabakkontrolle/download/PITOC/PITOC_Additives_in_Tobacco_Products_Report.pdf

National Institute for Public Health and the Environment in The Netherlands (RIVM) (2012). Tobacco additives. Information for Professionals. http://www.rivm.nl/dsresource?objectid=rivmp:185755&type=org&disposition=inline&ns_nc=1

New Zealand Ministry of Health Letter from British American Tobacco New Zealand to New Zealand Ministry of Health dated on 29 January 2014 <http://www.health.govt.nz/system/files/documents/pages/tobacco-returns-2013-bat.pdf>

Nonnemaker, J., Hersey, J., Homsy, G., Busey, A., Allen, J., Vallone, D. (2013). Initiation with menthol cigarettes and youth smoking uptake, *Addiction* 108, 171–178.

Noorafshan, A., Erfanizadeh, M., Karbalay-Doust, S. (2014). Sodium benzoate, a food preservative, induces anxiety and motor impairment in rats. *Neurosci Riyadh.* 19, 24–28.

Noriyasu, A., Konishi, T., Mochizuki, S., Sakurai, K., Tanaike, Y., Matsuyama, K., Uezu, K., Kawano, T. (2013). Menthol-enhanced cytotoxicity of cigarette smoke demonstrated in two bioassay models. *Tob. Induc. Dis.* 11(1), 18.

NTP (1993). Toxicology and carcinogenesis studies of 3,4-dihydrocoumarin (CAS. no. 119-84-6) in F344/N rats and B6C3F1 mice (gavage studies). September 1993. NTP-TR 423. NIH Publication no. 93-3154.

OECD (2010). OECD Guidelines for the Testing of Chemicals. Test Guideline 487.

OECD Chemicals Database Sids Initial Assessment Profile, Lactic Acid, CAS No. 50-21-5, Assessed Via CDG, <http://webnet.oecd.org/HPV/UI/handler.axd?id=fd79fce6-c7e2-48ed-aead-8728c961980c>

OECD SIDS, Benzaldehyde, CAS N°: 100-52-7, UNEP PUBLICATIONS <http://www.inchem.org/documents/sids/sids/100527.pdf>

OECD SIDS, Citric Acid, CAS N°:77-92-9, UNEP PUBLICATIONS <http://www.inchem.org/documents/sids/sids/77929.pdf>

Opdyke, D.L.J. (2013). Monographs on Fragrance Raw Materials: A Collection of Monographs Originally Appearing in Food and Cosmetics Toxicology. Elsevier, 750 p.

Ostrovskii, M.M. (1964). Hygiene and Sanitation 29,105-108.

Pang, X. Lewis, A.C. (2011). Carbonyl compounds in gas and particle phases of mainstream cigarette smoke. Science of the total environment 409, 5000-5009.

Philip Morris International SA (PMI) What's in Our Products? Company's website http://www.pmi.com/eng/our_products/pages/technical_products_information.aspx

Pierce, JS., Abelmann, A., Spicer, LJ., Adams, RE., Finley, BL. (2014) Diacetyl and 2,3-pentanedione exposures associated with cigarette smoking: implications for risk assessment of food and flavoring workers. Crit Rev Toxicol.;44(5), 420-35.

Pongsavee, M. (2015).Effect of Sodium Benzoate Preservative on Micronucleus Induction, Chromosome Break, and Ala40Thr Superoxide Dismutase Gene Mutation in Lymphocytes. BioMed Res Int Article ID 103512.

PubChem, Open Chemistry Database, Compound Summary for CID 244 benzyl alcohol, http://pubchem.ncbi.nlm.nih.gov/compound/benzyl_alcohol#section=Top

PubChem, Open Chemistry Database, Substance Record for SID 24901212 (+/-)-3,7-Dimethyl-1,6-octadien-3-ol
<http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=24901212#x332>

Pubchem, Compound Summary for CID 460, <http://pubchem.ncbi.nlm.nih.gov/compound/guaiacol#section=Top>

Purkis, S.W., Mueller, C., Intorp M. (2011). The fate of ingredients in and impact on cigarette smoke. Food and Chemical Toxicology 49 (12), 3238-3248.

Rabinoff, M., Caskey, N., Rissling, A., Park, C. (2007).Pharmacological and Chemical Effects of Cigarette Additives. American Journal of Public Health 97(11), 1981-1991. |

Redd, W.H., Manne, S.L., Peters, B., Jacobsen, P.B., Schmidt, H. (1994). Fragrance administration to reduce anxiety during MR imaging. J Magn Reson Imaging. 4, 623-626.

Renne R.A., Yoshimura H, Yoshino K, Lulham G, Minamisaw S, Tribukait A, Dietz DD., Lee KM, Westerberg R. B (2006) Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats Inhalation Toxicology: International Forum for Respiratory Research 18: 685-706

RIFM Expert Panel, Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.M., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H. (2007). A toxicologic and dermatologic assessment of ionones when used as fragrance ingredients.Food Chem Toxicol. 45 Suppl 1, 130-167.

Roemer E. Schorp, M,K. Piadé, J-J, Seeman, J.I, Leyden, D.E. and Hans-Juergen Haussmann Scientific assessment of the use of sugars as cigarette tobacco ingredients: A review of published and other publicly available studies. 2012. Critical Reviews in Toxicology 42(3): 244-278

Sax, N.I. (1984). *Dangerous Properties of Industrial Materials*. 6th ed. New York, NY: Van Nostrand Reinhold, 1984, p. 1327.

SCCP (2005) Scientific Committee On Consumer Products Opinion on Benzoic Acid and Sodium Benzoate SCCP/0891/05. Available at: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_015.pdf

SCCS Scientific Committee on Consumer Safety (2012) OPINION on Fragrance allergens in cosmetic products SCCS/1459/11

SCCS (Scientific Committee on Consumer Safety), Opinion on 56 titanium dioxide (nano form), 22 July 2013, revision of 22 April 2014.

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Addictiveness and Attractiveness of Tobacco Additives, 12 November 2010.

Scollo, MM, Winstanley, MH. *Tobacco in Australia: Facts and issues*. Melbourne: Cancer Council Victoria; 2015. Available from www.TobaccoInAustralia.org.au.

Siems, W., Salerno, C., Crifò, C., Sommerburg, O., Wiswedel, I. (2009). β -Carotene Degradation Products – Formation, Toxicity and Prevention of Toxicity). *Forum Nutr.* Basel, Karger, 2009, vol 61, pp 75–86.

Smith, S.S., Fiore, M.C., Baker, T.B. (2014) Smoking cessation in smokers who smoke menthol and non-menthol cigarettes. *Addiction*. 109(12), 2107-17. doi: 10.1111/add.12661. Epub 2014 Jul 21.

Sokol, N.A., Kennedy, R.D., Connolly, G.N. (2014) The role of cocoa as a cigarette additive: opportunities for product regulation. *Nicotine Tob Res*. 16(7), 984-991.

Stanfill, S.B., Ashley, D.L. (2000). Quantitation of flavor-related alkenylbenzenes in tobacco smoke particulate by selected ion monitoring gas chromatography-mass spectrometry. *J Agric Food Chem*. 48, 1298-306.

Stich, H.F., Rosin, M.P., Wu, C.H., Powrie, W.D. (1981). Clastogenicity of furans found in food, *Cancer Lett*. 13, 89-95.

Stone, V. (2012). Induction of micronuclei in cultured human peripheral blood lymphocytes. beta-Damascone. Covance Laboratories Ltd. Study no. 8240843. March 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Stotesbury, S., Digard, H., Willoughby, L., Couch, A. (1999). The pyrolysis of tobacco additives as a means of predicting their behavior in a burning cigarette. *Beiträge zur Tabakforschung / Contributions to Tobacco Research* 18 (4), 147–163.

Takasuka, N., Takahashi, M., Hori, Y., Kitahashi, T., Iigo, M., Imai, T., Yoshimi, N., Sugimura, T., Wakabayashi, K. (2009). Promotion of mouse two-stage skin carcinogenesis by diacylglycerol-rich edible oil. *Cancer letters* 275(1), 150-157.

Talhout, R., Opperhuizen, A., van Amsterdam, JG. (2006). Sugars as tobacco ingredient: Effects on mainstream smoke composition. *Food Chem Toxicol*. 44(11), 1789-1798.

The Good Scents Company Information System. <http://www.thegoodscentscompany.com/data/ex1021901.html>

The Hazardous Substance Data Bank (HSDB), The National Library of Medicine (NLM) 2004.

Touey, G.P., Kiefer, J.E. (1962). Patent: Bonding plasticizers for cigarette filters of cellulose acetate fibers: US 3393684 A.

Toxnet: <http://toxnet.nlm.nih.gov/>

TPSAC (2011). Tobacco Products Scientific Advisory Committee. Menthol Cigarettes and Public Health: Review of the Scientific Evidence and Recommendations. Rockville, MD: Center for Tobacco Products, Food and Drug Administration.

U.S. Food and Drug Administration (FDA), SCOGS (Select Committee on GRAS Substances) Database, GRAZ Substance, <http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogslisting&id=241>

U.S. Food and Drug Administration (FDA), CFR - Code of Federal Regulations Title 21 (2014)
<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1901>

U.S. National Library of Medicine, HAZMAP, 4-Acetylanisole
<http://hazmap.nlm.nih.gov/category-details?table=copytblagents&id=11064>

U.S. National Library of Medicine, Toxicology Data Network (TOXNET), 4-Acetylanisole
<http://chem.sis.nlm.nih.gov/chemidplus/rn/100-06-1>

U.S. National Library of Medicine, Toxicology Data Network (TOXNET), Piperonal CASRN: 120-57-0
<http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+581>

U.S. National Library of Medicine, Toxicology Data Network (TOXNET), Benzyl Alcohol CASRN: 100-51-1
<http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+46>

U.S. National Library of Medicine, Toxicology Data Network (TOXNET), Linalool CASRN: 78-70-6
<http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+645>

U.S. National Library of Medicine, Toxicology Data Network (TOXNET), The Carcinogenic Potency Project, Sorbic acid CAS 110-44-1
<http://toxnet.nlm.nih.gov/cpdb/chempages/SORBIC%20ACID.html>

U.S. National Library of Medicine, Toxicology Data Network (TOXNET), Summary Table by Chemical of Carcinogenicity Results in CPDB on 1547 Chemicals,
<http://toxnet.nlm.nih.gov/cpdb/pdfs/ChemicalTable.pdf>

University of California, San Francisco. Legacy Tobacco Documents Library. Chemical information file of Acetophenone, <http://legacy.library.ucsf.edu/tid/sgp95d00/pdf>

Valley Fig Growers. <http://www.valleyfig.com/about-our-figs>

Vanscheeuwijck, P.M., Teredesai, A., Terpstra, P.M., Verbeeck, J., Kuhl, P., Gerstenberg, B., Gebel, S. & Carmines, E.L. (2002). Evaluation of the potential effects of ingredients

added to cigarettes. Part 4: Subchronic inhalation toxicity. *Food and Chemical Toxicology*, 40, 113–131.

Wackowski, O.A., Delnevo, C.D. (2015). Young Adults' Risk Perceptions of Various Tobacco Products Relative to Cigarettes: Results From the National Young Adult Health Survey. *Health Educ. Behav.* (Epublication ahead of printing).

Wada, S., Hirose, M., Takahashi, S., Okazaki, S., Ito, N. (1990). para-Methoxyphenol strongly stimulates cell proliferation in the rat forestomach but is not a promoter of rat forestomach carcinogenesis. *Carcinogenesis* 11, 1891-1894.

Wayne, G.F., Carpenter, C.M. (2009). Tobacco industry manipulation of nicotine dosing. *Handb Exp Pharmacol* 192, 457-85.

Wayne, G.F., Henningfield, J.E. (2008). Tobacco product attractiveness as a contributor to tobacco addiction and disease. *Tobacco Attractiveness Report to Health Canada 2008*; 1-37.

Whitwell, J. (2012). Induction of micronuclei in cultured human peripheral blood lymphocytes. *Maltol. Covance Laboratories Ltd, England. Study no.8256119. May 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.*

Winter K, Barton D. The thermal decomposition of benzoic acid. *Can J Chem.* 1970;48(24):3797–801.

WHO (1967). The Eleventh Report of the Joint FAO/WHO Expert Committee on Food Additives 44; WHO techn.rep.ser. 383.

WHO (2002). Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives.

Willems, E.W., Rambali, B., Vleeming, W., Opperhuizen, A., van Amsterdam, J.G. (2006). Significance of ammonium compounds on nicotine exposure to cigarette smokers. *Food Chem Toxicol*, 44, 678-688.

Winter K, Barton D. (1970). The thermal decomposition of benzoic acid. *Can J Chem.* 48(24), 3797–801.

World Health Organization (WHO), Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2001.

Wu, Y., Yang, L., Liu, F., Miao, M., Zhu, H., Mao, D. (2007). Investigation of the pyrolysis behavior of 4-oxo-beta-damascone by on-line pyrolysis gas chromatography-mass spectrometry. *Se Pu Chin J Chromatogr Zhongguo Hua Xue Hui.* 25(3), 408–412.

Yılmaz, S., Ünal, F., Yüzbaşıoğlu, D. (2009). The in vitro genotoxicity of benzoic acid in human peripheral blood lymphocytes. *Cytotechnology* 60(1-3), 55–61.

Yin, C., Xu, Z., Shu, J., Wang, H., Li, Y., Sun, W., Zhou, Z., Fang M.C. (2014). Study on the effect of potassium lactate additive on the combustion behavior and mainstream smoke of cigarettes *J Therm Anal Calorim* 115, 1733–1751.

Yip, H, Taylor, L.T., Ashraf-Khorassani, M, Yu, J., Borgerding, M.F., Coleman III, W.A, and Bodnar, J.A. (2010). HPLC-MS Determination of Acrolein and Acetone Generated

from ^{13}C -Labeled Glycerol Added to Cigarette Tobacco Using Two Machine-Smoking Regimes. *Contributions to Tobacco Research* 24(2), 2; 48-57.

Zengin, N., Yüzbaşıoğlu, D., Ünal, F., Yılmaz, S., Aksoy, H. (2011). The evaluation of the genotoxicity of two food preservatives: Sodium benzoate and potassium benzoate. *Food Chem Toxicol.* 49(4), 763–769.

Zhao, Y., Fu, Y., Guo, Q.X. (2012) Production of aromatic hydrocarbons through catalytic pyrolysis of γ -valerolactone from biomass. *Bioresour Technol.* 114, 740-744.

Annex 1: Additives evaluated by the EU project 'Public Information Tobacco Control (PITOC)



Annex 2: Results of the literature search carried out by external company

The specification divided the searches required into two groups. The first group begins with tobacco or cigarette additives. The following keywords were suggested:

- Tobacco additives OR cigarette additives
- Tobacco additives OR cigarette additives AND toxicity/cancer/mutagenicity/reproductive toxicity
- Tobacco additives OR cigarette additives AND attractiveness/ addictiveness
- Tobacco additives OR cigarette additives AND flavour OR flavouring properties
- Tobacco additives OR cigarette additives AND inhalation
- Tobacco additives OR cigarette additives AND pyrolysis

Preliminary searches were carried out using PubMed in order to obtain an indication of the likely numbers of hits to be reviewed. The results were as follows:

- 1 - (Tobacco OR cigarette) - no restrictions 121161
- 2 - Results from 1 AND published since 1/1/2012 - 20442
- 3 - Results from 2 AND additive(s) - 180

As a result of the small number of hits, we did not carry out searches with the more specific terms but instead reviewed all of the results from the third search.

The second group begins with a number of identified additives: carob bean extract, cellulose fibre, guar gum, liquorice, menthol, prune juice concentrate, vanillin, 2-furfural, ammonium compounds, cocoa, glycerol, propylene glycol, sorbitol, sugars, acetaldehyde. The following key words were suggested to be used along with the additive name: toxicity/ attractiveness/ addictiveness/ flavour/ inhalation.

Preliminary searches using PubMed were carried out to obtain an indication of the numbers involved. Searches were initially carried out of each of the identified additives using only the additive name. Depending on the number of hits obtained, the results were refined by adding in turn the date restriction (1/1/2012 to present), then the "effects" terms ((toxicity) OR (attractiveness) OR (addictiveness) OR flavour) OR (inhalation)), and finally the additional terms ((cigarette) OR (tobacco)). The numbers of hits are shown in the table below.

Term	No restrictions	Time period	Terms	Additional terms
Carob bean extract	27			
Cellulose fibre	34			
Guar gum	1248	213		
Liquorice (includes liquorice)	3184	575	64	
Menthol	2291	457	60	

Additives used in tobacco products

Prune juice	15			
Vanillin	1825	447	54	
Furfural	2088	639	128	
Ammonium	71436	8979	547	1
Cocoa	3501	766	51	
Glycerol	61510	5715	581	9
Propylene glycol	4215	618	87	
Sorbitol	19659	1398	298	
Sugars	1274062	111253	20271	93
Acetaldehyde	9670	942	126	

Notes: Time period – published from 1/1/2012 to 30/1/2015
 Terms – ((toxicity) OR (attractiveness) OR (addictiveness) OR flavour) OR (inhalation)) AND with previous terms
 Additional terms – ((cigarette) OR (tobacco)) AND with previous terms

The results from the most refined search were reviewed for each of the additives in the table above. This gave a total of 1406 results to be reviewed. In addition, we skimmed the results from the “Terms” searches for ammonium and glycerol in case there are toxicity-related results which were excluded by using the additional terms. We did not do the same for the other additive for which the additional terms were used, the sugars, as the number of hits is too great.

The review of the results from the searches for individual additives showed few relevant papers for most of the additives. In order to widen the searches, further searches on PubMed were conducted using the name of the additive, the time period from 1/1/2012 and ((tobacco) OR (cigarette)). Revised searches were not conducted for carob bean extract, cellulose fibre, guar gum or prune juice as the original searches for these were not limited (or in the case of guar gum were only time limited). The numbers of hits are in the table below. These include any duplication with the original searches.

Additive	No of hits
Liquorice (includes licorice)	3
Menthol	107
Vanillin	7
Furfural	4
Ammonium/ammonia	65

Additives used in tobacco products

Cocoa	9
Glycerol	33
Propylene glycol	33
Sorbitol	7
Sugars	532
Acetaldehyde	37

In addition to the PubMed searches, more general web searches were carried out for relevant documents, using Google and Google Scholar. These began with either tobacco additives, or the name of each of the listed additives, and followed links as appropriate. There were a large number of results for the initial terms, but only the first 40-50 included any of relevance. The web searches were carried out after review of the PubMed results, so that we could make use of anything learned from the review, and recognise results already obtained and so reduce the effort required.