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WP9: D9.3 Report on the peer review of the enhanced reporting information on priority additives

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JOINT ACTION ON TOBACCO CONTROL

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Chapter 1. Introduction

1.1 Background

The new Tobacco Products Directive (TPD) 2014/40/EU strengthens the rules regarding the reporting and composition of tobacco products. In addition to tighten the obligations of manufacturers to report on ingredients contained in tobacco products in general, enhanced reporting obligations apply to 15 priority additives added to cigarettes and roll-your-own (RYO) tobacco by May 2016. The list of priority additives has been developed by the European Commission (EC) based on previous assessment by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR).

For these priority additives, comprehensive studies have to be carried out by the industry which shall examine for each additive whether it has any of the properties a) to d) specified in Article 6.2 of the TPD:

"Member States shall require manufacturers and importers of cigarettes and roll-your-own tobacco containing an additive that is included in the priority list provided for in paragraph 1, to carry out comprehensive studies, which shall examine for each additive whether it:

(a) contributes to the toxicity or addictiveness of the products concerned, and whether this has the effect of increasing the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;

(b) results in a characterizing flavor;

(c) facilitates inhalation or nicotine uptake; or

(d) leads to the formation of substances that have CMR properties, the quantities thereof, and whether this has the effect of increasing the CMR properties in any of the products concerned to a significant or measurable degree."

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 role of the independent review panel is described as follows in article 6.4 of the TPD: *"The Commission and the Member States concerned may require these reports to be peer reviewed by an independent scientific body, in particular as regards their comprehensiveness, methodology and conclusions. The information received shall assist the Commission and Member States in taking the decisions pursuant to Article 7."*

In line with this, an independent review panel consisting of 10 scientific experts in various relevant fields was established. In order to guide the tasks of the independent review panel, an assessment/ evaluation framework was created (D 9.1) prior to the reception of the industry reports. As the submitted studies followed a different structure than anticipated, the approach suggested in the framework was revised during the work of the independent review panel.

1.2 Industry reports

The reports submitted to the European Union Member State (EU MS) regulators contained the results of literature searches, smoke chemistry assessment, toxicity studies, human clinical studies and a sensory analysis assessment of each additive, performed by subcontracted companies. These reports were submitted under the umbrella of a Priority Additives Tobacco Consortium of 12 international tobacco companies, to which subcontracted companies provided study results.

1.3 Panel and Review procedure

To facilitate peer review of the enhanced reporting information on priority additives, an independent review panel of international experts of various relevant expertise was established. These reviewers worked together with several participants from WP 9 partner institutes. The members of the review panel, and the partners associated with WP9 task 2.4, are:

Review Panel

Dr. S. Caillé-Garnier	Université de Bordeaux, France
Dr. F. Henkler-Stephani	German Federal Institute for Risk Assessment (BfR), Germany
Dr. A. K. Bolling	Norwegian Institute of Public Health (NIPH), Norway
Dr. C. Lambré	Consultant, France
Dr. C. Michel	French agency for food, environment and occupational health & safety (ANSES), France
Dr. J. Pennings	National Institute for Public Health and the Environment (RIVM), The Netherlands
Dr. P. Schwarze*	Norwegian Institute of Public Health (NIPH), Norway
Dr. J-P. Tassin	Sorbonne University, France
Dr. C. Vardavas*	Hellenic Cancer Society (HCS), Greece
Dr. E. Zervas	Hellenic Thoracic Society and Hellenic Open University, Greece

WP 9 task 2.4 partners

National Institute for Public Health and the Environment (RIVM), The Netherlands
Dr. A. Havermans, Dr. L. van Nierop, Drs. C Pauwels
German Federal Institute for Risk Assessment (BfR), Germany
Dr. T. Schulz, N. Mallock
French agency for food, environment and occupational health & safety (ANSES), France
Dr. T. Mansuy
Italian National Institute of Health (ISS), Italy
Dr. R. Solimini
Andalusian Agency for Agriculture and Fisheries (AGAPA), Spain
M. Merino

* Individuals marked with an asterisk are both member of the review panel and involved through WP 9 partner institutions (i.e. as "supporting staff").

Process

The review work was carried out by the panel in collaboration with the partner institutes that had hours attributed for task 2.4 in work package 9 (RIVM, BfR, ISS, ANSES, HCS, NIPH, AGAPA). The participants from these institutes (i.e. "supporting staff") were asked to write a first draft of parts of the reports. That is, for each additive, the writing of sections on chemistry, toxicity and addictiveness was attributed to "supporting staff" with relevant expertise. In addition, per additive, one partner institute was appointed as leading institute and was responsible for combining all separate parts into one draft report and adding general sections. Complete draft reports for the individual additives were then circulated to the review panel and critically reviewed. The results of this review were discussed in group-conference calls (with the review panel and "supporting staff") and processed by the leading institute, with help of the relevant supporting institute(s), if needed.

After drafting all reports, the outcomes were discussed in a physical meeting that took place following the JATC interim meeting (February 2020). After this meeting, outcomes were drafted in Chapter 2 of this deliverable and reviewed by the review panel. Moreover, individual reports were

critically evaluated and restructured and an additional evaluation of main stream smoke chemistry was performed (see Section 3.3). Methodological considerations and limitations were gathered throughout the review process and described in Chapter 4. More information about the review procedure and decisions that were taken in the process, can be found in Chapter 3.

1.4 Overview of the deliverable

This WP 9 deliverable includes 6 Chapters and 4 Annexes. In Chapter 2, the outcomes and conclusions of the review panel that reviewed the industry reports are presented. Information about the review procedure and decisions taken in the process, can be found in Chapter 3. During the review of the industry reports, the panel came across shortcomings in the approach and the applied methodology that were common to all additive reports. These are described in Chapter 4. Finally, Chapter 5 contains the 15 individual additive reports drafted by WP 9 and the review panel. Extra information concerning the additives can be found in the Annexes. Annex I shows an outcome table which provides an overview of the outcomes from the industry studies according to the industry and according to the review panel (in line with what is described in Chapter 2). An overview of carbonyl emissions extracted from the industry reports and analyzed by the review panel can be found in Annex II. An overview of pyrolysis products and their possible CMR properties as assessed by the review panel can be found in Annex III. Lastly, Annex IV addresses the Priority Additives Tobacco Consortium's response (September 2019) to an initial letter from the review panel (June 2019) that raised methodological concerns and requested complementary information.

Chapter 2. Outcomes and conclusions

A consortium of 12 tobacco manufacturers (British American Tobacco (Investments) Limited, Imperial Tobacco Limited, JT International SA, Philip Morris Products SA, KT&G Corporation, Joh. Wilh. Von Eicken GMBH, Karelia Tobacco Company Inc., Landewyck Tobacco S.A., Mac Baren Tobacco Company A/S, Pöschl Tabak GMBH & Co. KG, Scandinavian Tobacco Group A/S and Continental Tobacco) has jointly submitted 14 reports: one for each priority additive, except for diacetyl. As described in Chapter 1, these reports have been submitted to scientific review by an independent review panel installed by Work Package 9 of the Joint Action on Tobacco Control. The panel has specifically reviewed the reports comprehensiveness, methodology and conclusions (as described in TPD article 6.4). In addition, the panel has evaluated the chemical, toxicological, addictive and flavoring properties of each of the priority additives, based on the information presented by the industry, supplemented with the review panel's expertise and knowledge of independent research. This Chapter 2 outlines the main outcomes of the review and conclusions from the review panel.

2.1 Quality of the Industry reports

2.1.1 Overall structure

The industry reports generally contain a summary, followed by chapters with overviews of the study designs and findings and many annexes with the detailed methodology and results. In general, the reports contain less than 200 pages of main text and over 2000 pages of appendices. The submitted reports are not clearly structured, with many crossing references to annexes and appendices. The numbering is not homogeneous and in some cases incorrect for the figures, tables and references. Several graphs and tables are not interpreted. References are split in different parts of the reports. Some of these are missing in conclusions, as are some footnotes and information. Appendices have appendices which themselves have appendices, and some of them are missing. This unclear structure significantly hindered the scientific evaluation of the provided studies.

2.1.2 Comprehensiveness

Although most of the relevant topics are covered in the industry reports, some important aspects of the assessment are missing for most of the additives. In addition, as described in Chapter 4, the quality of the reports is not sufficient and therefore we conclude that they are not comprehensive. The insufficient quality was both due to limitations in the overall approach chosen in the industry reports and a range of specific methodological limitations.

Examples of aspects that were not addressed or were of insufficient quality:

There are very few independent studies included in the industry literature review, even though there are many more independent studies on relevant topics available and retrieved by the panel. The list of analytes applied in the chemical comparative experiments did not include the pyrolysis products identified in the literature review.

- Inhalation toxicity is not evaluated for any of the additives, while this is a highly relevant toxicological outcome for additives applied in products that are smoked.
- Toxic, genotoxic and carcinogenic potential of the products formed by pyrolysis is not assessed. This includes the irritant effect of many of them, which may foster the toxicity of tobacco ingredients.
- The provided clinical study was not sufficient to address concerns regarding addictiveness of additives
- The effect of sugars potentiating the addictive effects of nicotine through the process of monoamine oxidase inhibition was not addressed.
- Although many additives may increase the attractiveness of cigarettes and RYO tobacco, for example by providing flavor, humidifying tobacco or reducing harshness of smoke, this aspect is not addressed in the industry reports.
- The characterizing flavor assessment is missing for some additives, also for additives known to have flavoring properties or have pyrolysis products with such properties (e.g. guar gum, maltol and sorbitol)
- An integrated discussion of new results from the industry laboratory studies and the conclusions based on the literature review is missing. Similarly, results from the comparative chemical analysis are not applied in the toxicological evaluation.

2.1.3 Methodology

The review panel concludes that the methodology is not of sufficient quality (see Chapter 4 for details), some important examples are:

- The overall approach seems to have been designed to show no effect/predetermined effect from the addition of priority additives on the chemistry, toxicology and addictiveness.
- The statistical approach applied to test the difference between the reference cigarettes and the products containing the additives has several serious limitations.
- Evaluation of the chemical compounds formed form the pyrolysis of the additives is confined to the five most abundant compounds and those included on the Hoffman list, even though there may be many more chemicals formed from pyrolysis that are not included on that list. Moreover, only data from literature were presented, without other updated experiments.
- The chemical compounds formed from the pyrolysis of the additives are not included in the comparative analyses between references and test cigarettes.
- The methodology used to assess characterizing flavor of the additives is flawed.

2.1.4 Conclusions

Since the review panel considered the quality of the reports and the applied methodology to be insufficient, the panel members question the scientific validity of the conclusions and

recommendations in the industry reports, and conclude that these are not warranted.

Although most of the relevant topics mentioned in the TPD were addressed in the industry reports, no scientifically valid conclusions can be drawn based on the submitted reports. Therefore, the panel concludes that the reporting obligations have not been fulfilled. In addition, some of the obligations from the TPD were not covered in the reports:

- Article 6.3. Interactions with other tobacco ingredients have not been studied

- Article 6.2. The industry reports do not cover the contribution of the additives to toxicity (e.g. toxicity of pyrolysis products or inhalation toxicity) or addictiveness. Note that the industry based their comparative testing approach for toxicity on Article 7, while the review panel based their review on Article 6 and consider the separate parts of 6.2.a as equivalent (for details, see Section 2.2 below).

2.1.5 Should more information be requested from the industry?

In the review panel's opinion, the industry should not be asked to provide further information, as we do not expect the industry to report back on such requests with sufficient scientific quality. This has become clear from the industry response received to a first letter indicating additional requirements from the review panel. The industry's response to this letter contains very little relevant additional information and has not affected the outcomes of the review of the industry reports (see Annex IV). Another illustrative example of the industry approach is that, their report on menthol does not mention the strong evidence from independent literature on menthol's capacity to facilitate inhalation and addictiveness. This shows a deliberate decision from the industry consortium to ignore existing evidence and bias their reports in favor of their own products.

The review panel concludes that placing the responsibility for assessing priority additives on the tobacco industry is not suitable. The industry has a clear motivation to keep their products on the market, and also to maintain sales numbers, and therefore cannot be considered an unbiased part. Instead, assessment of additives in tobacco products should be based on independent sources and be performed by independent experts. This conclusion from the review panel is in line with findings from previous consortium groups assessing industry data on additives and tobacco products e.g. (1, 2). Moreover, it reflects EU practice when it comes to addition of chemicals in food and other products, as these are evaluated by independent organs such as EFSA and ECHA.

2.2 Interpretation and ambiguity of the TPD

The interpretation of the TPD presented in the industry reports differs from the review panels' interpretation of the TPD. These different interpretations appear to be due to the ambiguity of the wording in Article 6.2.a and the conflicting content of the Articles 6.2.a and 7.9. A revision of these paragraphs seems to be required for an unambiguous interpretation of the TPD.

Article 6.2.a states that the industry reports need to assess whether an additive a) *contributes* to the toxicity or addictiveness of the products concerned, and b) whether this has the effect of *increasing* the toxicity or addictiveness of any of the products concerned to a significant or measurable degree. The review panel interpreted these as two independent sentences, that require evidence for both a) the additive *contributing* to the endpoint studied, and b) the additive *increasing* the effect size of the endpoint studied. In contrast, in the industry reports, the second part of the sentence is interpreted as the primary assessment criterion and only evidence for the additives b) *increased* effect size of the endpoint in question is addressed.

Article 7.9 requires regulatory actions by the member states if the additives *"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". Thus, any regulatory actions of the member states can only be based on the second part of Article 6.2.a regarding increased toxicity. The only current experimental approach to assess this aspect of an additive's toxicity is comparative testing, which lacks discriminating power in the case of products with an extremely high toxicity such as cigarettes (3). Since there are currently no experimental tests available to provide evidence that tobacco additives increase tobacco toxicity to a significant degree, the current phrasing of the TPD will not allow member states to take actions based on the toxic properties of the additives.

Article 6 describes the required content of the industry reports and the independent review panel, and also states that the information received from the review panel *"shall assist the Commission and Member States in taking the decisions pursuant to Article 7"*. As described above, the review panel's interpretation of the TPD is that the work of the review panel should be based on Article 6, and not on Article 7. In contrast, the industry chose to base the of majority their reports on comparative testing, and use Article 7.9 as an argument for this approach, without evaluating if this comparative testing was sensitive enough for a scientifically valid assessment.

2.3 Concerns regarding additives

The conclusions presented in the reports provided by the industry consortium suggest that none of the additives, in the current application levels, are associated with concern when used in cigarettes or RYO tobacco. The data and conclusions provided by the industry are listed in Table A.1 for each additive, together with the review panel's assessment of the provided (and independent) data (see Annex I). As described above, the review panel members conclude that the conclusions and recommendations in the industry reports were not warranted, due to the poor quality of the submitted reports. In spite of the limitations, the panel has pointed out concerns regarding specific additives based on their identified properties, based on the review panel's expertise and knowledge of independent research. The specific concerns pointed out by the review panel below are all related to specific sections of the TPD. Please note that the concerns pointed out do not reflect an exhaustive list of possible concerns associated with application of additives in cigarettes or RYO tobacco.

The selection of additives listed below does not imply that additives not mentioned are safe when used in cigarettes or RYO tobacco. The list reflects the additives for which sufficient knowledge was available within the shared knowledge of the review panel, i.e. they were "low hanging fruits". A full review of independent literature for all of the additives was not possible within the allocated resources and time-frame of work requested from the panel members. Consequently, each member state (MS) must consider whether the information provided by the review panel is sufficient to ban an additive.

2.3.1 Facilitation of inhalation

There is strong evidence from independent literature that menthol facilitates inhalation by activating the cooling receptor TRPM8. All menthol analogs, including geraniol, have the same agonistic effect on this receptor and can work synergistically. Therefore, tobacco and related products containing menthol and its analogs, do not comply with Article 7 (6d) of the European Tobacco Product Directive 2014/40/EU.

Since May 2020, cigarettes and RYO products containing menthol as a characterizing flavor are prohibited based on the Tobacco Products Directive (TPD art. 7.1). However, the TRPM8-dependent cooling effect -that facilitates inhalation of irritating fumes- occurs already at levels far below the threshold of characteristic aroma properties. Importantly, this is an intrinsic property of menthol and does not comply with Article 7 (6d) of the TPD, even if application levels in tobacco might not induce measurable effects. Therefore prohibiting the addition of menthol at all application levels is advised. Some member states, such as Germany and Finland, currently prohibit application of menthol at any application level based on its inhalation facilitating properties.

Continued

2.3.2 Carcinogenicity

The European Commission has classified **titanium dioxide** as a "carcinogen category 2", the classification becomes effective on September 9th, 2021 (4). The classification applies only to mixtures in powder form containing 1 % or more of titanium dioxide in the form of or incorporated in particles with aerodynamic diameter ≤ 10 µm. Titanium dioxide is currently used in filters in some commercial products, and transfer to main stream smoke was not adequately assessed in the industry report. Due to the lack of data demonstrating that that there are no titanium dioxide particles in smoke, from that date, tobacco and related products containing titanium dioxide do not appear to comply with Article 7 (6e) of the European Tobacco Product Directive 2014/40/EU.

2.3.3 Addictiveness

Combustion of sugars produces carbonyls, some of which potentiate addictive effects of nicotine through the mechanism of Monoamine Oxidase Inhibition (MAOI) (5, 6). This was also indicated in the 2010 SCENIHR report and acknowledged at the WHO workshop on addictiveness in 2018 (WHO, Berlin, Germany 15-16 May 2018)(7, 8). Limits for sugars and sugar-related compounds may however be difficult to enforce, as the industry has much flexibility to use different individual compounds, including complex carbohydrates or sugar alcohols to circumvent possible regulations. A more promising approach, as suggested by the WHO Study Group on tobacco product regulation (TobReg), would be to set limits for aldehydes (and other toxicants) yields per cigarette (9). Pennings *et al.* demonstrated that formaldehyde yields are in particular useful to monitor emissions of other toxicants, including carbonyls (10). Further, it is advised for regulators to consider sugars and humectants as targets for future tobacco regulation, consistent with previous proposals by TobReg (9). It is therefore recommended that the commission and member states consider whether mandatory limits for selected carbonyls are appropriate measures to restrict the effects of sugars and related additives, such as **sorbitol**, on the addictiveness (and attractiveness) of tobacco products. In addition, mandatory reporting obligations on aldehyde levels in smoke would be essential for adequate risk assessment. For **guar gum** and **sorbitol**, the data provided by the industry, showing increased levels of carbonyls for increasing levels of additive, are sufficient to support further action (Table A.1 – Annex I).

2.3.4 Reporting obligations not fulfilled

For **diacetyl**, no report was provided by the industry. Therefore, any cigarette and roll your own products containing diacetyl do not comply with Article 6 of the European Tobacco Product Directive 2014/40/EU.

2.3.5 Flavoring additives and attractiveness

The issue whether additives can lead to a characterizing flavor is complex and should be decided in relation to its use in a particular product by the expert panel installed by the EC, according to Decision (EU) 2016/786. According to the assessment provided by the industry consortium, none of the additives provided a characterizing flavor to cigarettes. However, there were methodological limitations in the assessment (see Section 4.9).

Moreover, many of the additives on the priority additive list, and/or their pyrolysis products, are known flavorings and/or sweeteners **(carob bean, cocoa, diacetyl, fenugreek, fig, geraniol, guaiacol,**

*The classification applies to titanium dioxide that in powder form contains 1 % or more of titanium dioxide which is in the form of or incorporated in particles with aerodynamic diameter ≤ 10 µm. There is currently no information regarding the size of particles of titanium dioxide applied in cigarettes provided by manufacturers in EU-CEG. We recommend that this information is requested from the industry to support enforcement.

guar gum, licorice, maltol, menthol, sorbitol). These substances modify the flavor of cigarettes, making them more attractive, especially for young and new users. The addition of flavorings making the cigarette attractive and palatable is in itself cause for concern. Additives that increase the attractiveness of tobacco products ultimately increase the risk for addiction by promoting the exposure of vulnerable populations such as teenagers, and increase tobacco-related diseases by encouraging initiation and repeated use.

The TPD also recognizes the relevance of attractiveness of tobacco products, as it is mentioned several times in the introduction and article 19.1 (a). However, quantitative evaluation and regulation of attractiveness are currently not covered by the TPD. Yet, additives with flavoring and other attractive properties are of concern according to the review panel and member states are advised to take this parameter into account in the evaluation of tobacco additives. Moreover, they may have the possibility to ban flavoring and/or other attractive additives in national regulation.

2.4 Publishing application limits

Any products containing additives in higher amounts than assessed in the industry reports do not comply with the European Tobacco Product Directive 2014/40/EU. To support enforcement of this regulation, Member States may publish the current maximum application levels on relevant websites. It should be emphasized that this does not imply that additive amounts below the set application levels can be considered safe.

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Chapter 3. Review panel approach

As summarized in Chapter 1, Article 6.2 of the TPD describes what aspects of the priority additives should be examined by manufacturers. However, the TPD does not give specific instructions regarding how this should be done, nor what the peer review process should comprise. This led to room for interpretation of several aspects of the work performed by the review panel. This Chapter describes the approach of the independent review panel, including the ambiguity and interpretation of the TPD, the corresponding approach for evaluation of the chemistry and toxicity data submitted by the industry, and the criteria for the independent evaluation of mainstream smoke (MSS) chemistry by the review panel.

3.1 Interpretation of the TPD and overall approach

3.1.1 Ambiguity of the TPD

During the review of the industry reports concerning the priority additives, it became clear that the interpretation of the TPD presented in the industry reports differed from the review panels' interpretation of the TPD. These different interpretations appear to be due to the ambiguity of the wording in Article 6.2.a and the conflicting content of the Articles 6.2.a and 7.9. and a revision of these paragraphs seems to be required for an unambiguous interpretation of the TPD. Reasons for this are outlined below.

- Firstly, the wording of Article 6.2.a is ambiguous. It states that the industry reports need to assess whether an additive a) *contributes* to the toxicity or addictiveness of the products concerned, and b) whether this has the effect of *increasing* the toxicity or addictiveness of any of the products concerned to a significant or measurable degree. The review panel interpreted these as two independent sentences, that require evidence for both a) the additive *contributing* to the endpoint studied, and b) the additive *increasing the effect size* of the endpoint studied. In the industry reports, the interpretation is different, and only evidence for the additives b) *increased effect size* of the endpoint in question is covered. Based on discussions within the panel and with legal experts, it seems difficult to draw a conclusion regarding which interpretation is correct. Thus, a revision of the phrasing of Article 6.2.a seems to be required to clear up the ambiguities.
- Secondly, Article 7.9 requires regulatory actions by the member states if the additives "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". Thus, it is clear that any regulatory actions of the member states can only be based on the second part of Article 6.2.a regarding increased toxicity. The only current experimental approach to assess this aspect of an additive's toxicity is comparative testing, which lacks discriminating power in the case of products with an extremely high toxicity such as cigarettes. Since there are currently no experimental tests available to provide evidence that tobacco additives increase tobacco toxicity to a significant degree, the current phrasing of the TPD will not allow member states to take actions based on the toxic properties of the additives. Here also, a review of the phrasing of Article 6.2.a and 7.9 of the TPD appears to be required for the wording to be more consistent and reflect the limitations of the current scientific methodology for the intended purpose.
- Finally, the TPD Article 6 describes the required content of the industry reports and the independent review panel, and also states that the information received from the review panel *"shall assist the Commission and Member States in taking the decisions pursuant to Article*

7”. The review panel’s interpretation of the TPD is that the work of the review panel should be based on Article 6 only, and not on Article 7. In contrast, the industry reports have chosen to use Article 7 as an argument for their comparative testing approach, and thus base their reports on both article 6 and 7.

In summary, the members of the review panel based their work on Article 6 only, and assessed evidence for a) the additive contributing to the endpoint studied, and b) the additive increasing the effect size of the endpoint studied.

3.2 Overall approach of the review panel

As the panel consists solely of scientists and no legal experts, conclusions presented are only based on a scientific perspective.

All panel members agreed that their primary task was to draw conclusions, on each additive’s properties according to article 6 in the TPD, based on the information submitted in the industry reports. Additionally, evidence from independent literature was used when available, but no comprehensive literature review was performed. If the panel members could not draw a conclusion based on the submitted information, the submitted information was considered insufficient. Even though the industry reports were often of insufficient quality, the reviewers chose to raise concerns for some additives, based on the provided information and independent literature.

The additives associated with concern reflect the additives where sufficient knowledge was easily available within the shared knowledge of the review panel, i.e. they were “low hanging fruits”. A full review of independent literature of the additives was not possible within the allotted resources and time-frame of work requested by the panel members. Consequently, each member state (MS) must consider whether the information provided by the review panel is sufficient to ban an additive. The selection of additives associated with concern in the report does not imply that additives where no concern is pointed out are safe when used in cigarettes or RYO tobacco.

In the review process, the individual additive reports were drafted by different researchers and institutions. Consequently, the extent of independent literature searches performed by reviewers and references to independent sources vary between the reports. Similarly, the level of detail in different sections vary between the individual additive reports, although the main sections are the same in each report.

3.3 Evaluation of chemistry and toxicity data

3.3.1 Assessment criteria for exposure

Evaluation of the chemistry and exposure data included in the industry reports requires a common understanding of the exposure resulting from inclusion of the additive in the tobacco. The two types of experiments performed to assess altered/increased exposure due to inclusion of an additive are chemical analysis of mainstream smoke (comparative testing) and emissions from pyrolysis experiments, as illustrated in Figure 3.1.

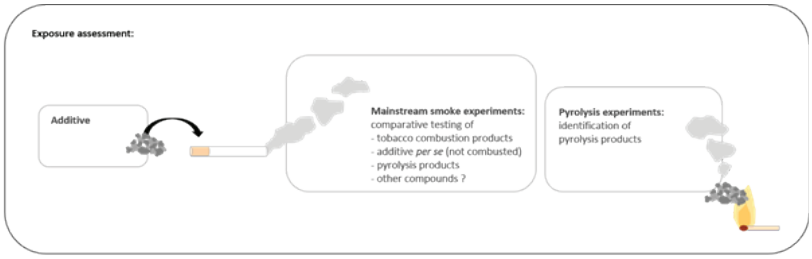

Figure 3.1: Schematic illustration of exposure assessment strategies.

According to the review panel, the information required from these experiments to evaluate the risk resulting from application of the additive in the tobacco includes:

- What is the transfer rate of the *additive itself* to the smoke? (using comparative testing)
- What are the *pyrolysis products* of the additive? (using pyrolysis experiments in well controlled laboratory experimental conditions (temperature, temperature increase rate, duration time, etc.) with pure additive)
- Are these *pyrolysis products* detected in mainstream or sidestream smoke? (using comparative testing)
- Does inclusion of the additive alter the levels of *tobacco combustion products or the formation of new ones?* (using comparative testing)
- Are the levels of *other compounds* (than the additives specific pyrolysis products and tobacco combustion products) increased by inclusion of the additive? (using comparative testing)

Whether and how the above information is provided by the industry is described in each additive report. However, it should be noted that in general, the information provided was not considered to be of sufficient quality by the review panel, as described in Chapter 4. For instance, the information regarding pyrolysis products was only based on literature, and the list of analytes in the comparative chemical analysis was not expanded with the pyrolysis products identified in the literature. In addition, the comparative testing approach had several methodological limitations, for instance the statistical approach was not in line with the methodology applied in the independent scientific literature.

Due to the limitations in the provided data, an independent evaluation of the results from the comparative testing was performed for carbonyls, as described below (see Section 3.4).

3.3.2 Assessment criteria for Toxicity

As illustrated in Figure 3.2, a selection of approaches may be applied in a toxicological evaluation of the consequences of inclusion of the additive in the tobacco. These include, but are not limited to:

A. Evaluation of the hazard properties of the **additive itself** based on existing information, in particular the oral route (**ingestion**) based on its application as a food additive. This should include evaluation of the general toxicity of the additive based on reports from regulatory bodies. Information regarding Acceptable Daily Intake (ADI) and the general exposure level of the population should be reported. This route of exposure is not appropriate for the evaluation of the toxicity of a compound as tobacco additive, but this information may provide some clues about a possible target organ, toxic effect and/or dose-response curves.

B. Evaluation of the **additive itself via inhalation** due to transfer of additive (non-combusted) in the smoke. Experiments and evaluations specific for inhalation should be included. In addition, toxicokinetic assessment following inhalation compared to oral route should be included. Evaluation of the toxicity of the metabolites formed following inhalation as compared to those formed form following ingestion is required. Finally, evaluation of the health risk, should consider the total exposure to the additive and its metabolites resulting from its uses in both food and tobacco products.

C. Evaluation of **pyrolysis products** (inhalation exposure route). Comparison of results from pyrolysis experiments and mainstream tobacco smoke experiments (comparative testing) is required to evaluate the exposure level of pyrolysis products. Then, assessment of toxicity of identified pyrolysis products is required. It would be feasible to include tobacco smoke as a reference material in these experiments.

D. Evaluation of **mainstream smoke** (inhalation exposure route). Comparative experiments to assess whether the additive contributes to increased toxicity of the generated smoke. This can be assessed either i) directly in terms of comparative toxicity testing or ii) indirectly by comparative

chemistry testing followed by toxicological evaluation of the significant changes in mainstream smoke chemistry. However, for the characterization of the additive's toxicity, the comparative testing approach is not sensitive enough and lacks discriminating power. Finally, the choice of methods for smoke generation and statistical analysis may introduce methodological limitations for both chemical and toxicological comparative testing (see Chapter 4).

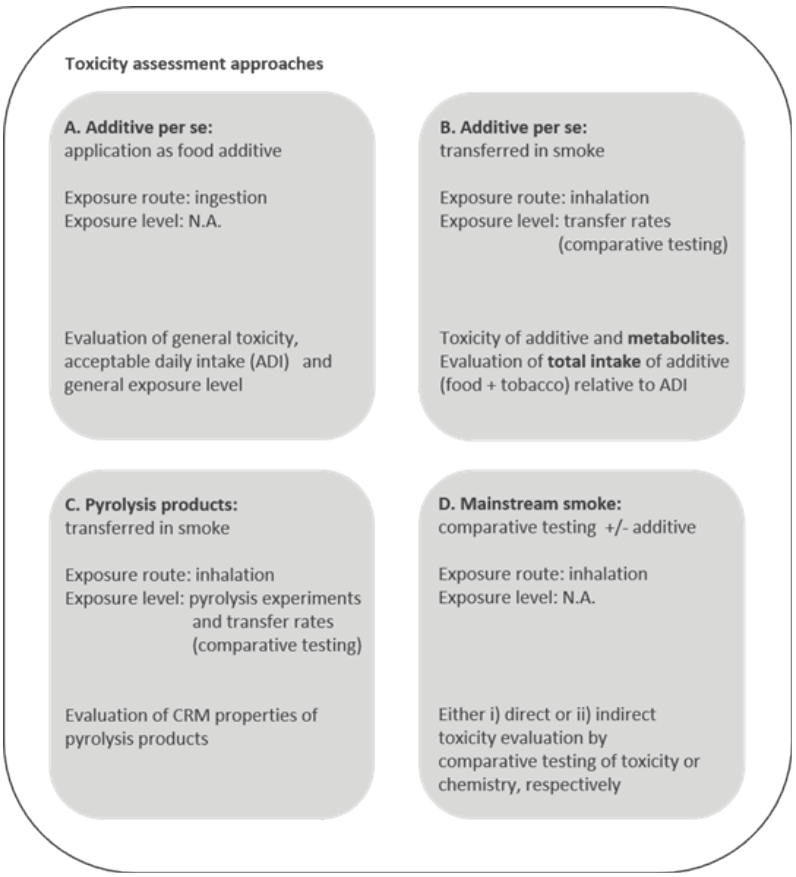

Figure 3.2: Schematic illustration of toxicity assessment strategies.

Whether these approaches were assessed or not in a satisfactory manner in the industry reports is described in the individual additive reports prepared by the review panel. In general, the industry reports focused on the toxicity of the additive per se and *in vitro* comparative toxicity testing (points A and D), while no data was presented for inhalation toxicity of the additive itself or its pyrolysis products (points B and C). The methodological limitations in the comparative testing approach are discussed in detail in Chapter 4.

3.4 Independent evaluation of mainstream smoke chemistry

The review panel identified serious limitations in the study design and the statistical approach of the comparative experiments of mainstream cigarette smoke provided by the industry (see Chapter 4). In order to interpret the results in spite of these limitations, the review panel performed its own, independent evaluation of the industry data. This in-depth evaluation of emission levels was focused on carbonyl compounds, as (in addition to their toxic properties) some of them, such as aldehydes, are known to inhibit monoamine oxidases (MAO) and thereby may increase tobacco addictiveness.

The limitations in the industry approach are described in depth in Chapter 4. In short, the comparative smoke experiments for determination of carbonyl compounds were performed in two separate sets. The first set of measurements (carob bean, cocoa, fenugreek, fig, glycerol, licorice, menthol) seems to have been performed under acceptable conditions, generally resulting in standard deviations of approximately 10%, in line with previous studies. The second set (geraniol, guaiacol, guar gum, maltol,

propylene glycol, sorbitol) resulted in much higher standard deviations. This implies inconsistencies in the laboratory procedures, and the review panel concluded that these results were of poor quality. With regard to the statistical approach, several choices were made by the industry that were likely to contribute to false negative results , including comparison with historical data in the statistical analysis and application of a 99% instead of 95% confidence criterion (see Chapter 4 for a more detailed description).

Since the data resulting from experimental set 2 were poor, and not suitable for a regular statistical analysis, a general stepwise approach was applied by the review panel for an independent evaluation of the carbonyl data. In spite of the poor data quality, this approach allowed for extraction of useful information from the data provided by the industry. The applied stepwise criteria were:

- **Does the additive affect the mainstream smoke chemistry?** To assess whether an additive affects the mainstream smoke chemistry, the mean values and standard deviations for test cigarettes containing three different levels of the additive and the additive-free reference items were compared. If the standard deviations for test and reference cigarette did not overlap, this was identified as a possible increase or decrease due to the additive. It must be noticed that, due to the poor quality of methodology used by the industry and the resulted data, an overlap does not necessarily means that there is no impact of the additive.
- **Is there a "dose-response" pattern?** Generally speaking, if an increase in the level of a chemical compound in the smoke is caused by the tested additive, the concentration of this compound in the smoke should, in most cases, increase with the application level of the additive in the test cigarette. For instance, if an increase was observed for acrolein for the test cigarette containing 0.015% geraniol, an even higher increase in acrolein would be expected for the test cigarette containing 0.045% geraniol. However, the pyrolysis/combustion mechanism is more complex and, in some cases, the presence of even low levels of additives can affect the physical and chemical properties of the tobacco and/or of the combustion process, resulting in non-linear or even reverse dose-response relationships. One example could be if two levels of additive results in small differences in the physical conditions, resulting in significant changes in the combustion process and thus in the emissions. Note that this criterion was only developed for the evaluation of the poor-quality data in the industry reports and should not be considered as a general criterion in evaluation of mainstream smoke chemistry.
- **Is the increase plausible?** In general, it is reasonable to assume that an additive used in cigarettes in low application levels (e.g. below 0.1%) is unlikely to significantly increase emission levels of major smoke constituents. However, as noted above, there can be exceptions where even low levels of additives alter the physicochemical properties of the tobacco. Another case is when the additive itself is a precursor of one or several smoke components. In the evaluation of the industry data, if an additive was found to affect the chemistry for one or two of the three tested application levels (Low, Max and Max plus), the review panel concluded that the observed effect on the smoke chemistry was not plausible if there was i) a lack of a "dose-response" pattern and ii) an additive application level < 0.1%.

Please note that this evaluation does not reflect a complete independent evaluation of effects of additives on mainstream smoke chemistry, as it is solely based on the data provided by the industry, which in several cases appeared to be of insufficient quality. The purpose of this independent evaluation by the review panel was to extract any information that could be extracted from the industry reports, rather than to dismiss the industry evaluation of mainstream smoke chemistry completely. It should be noted that, if the independent evaluation did not identify an increase in any of the compounds evaluated by the industry, this does not reflect that the increase does not occur and an additive can be considered as safe. The results of the independent evaluation are presented in Annex II, and were used in the evaluation of the individual additives presented in Chapter 6.

A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

Chapter 4. Methodological considerations and limitations of industry reports

During evaluation of the industry reports, the review panel has identified several limitations and insufficiencies in the industry's approach and methodology. The encountered limitations are summarized and discussed within this Chapter. To make this Chapter 4 comprehensible by itself, some information from other parts of this document might be repeated with the same or similar wording. First we briefly present the approach chosen by the industry, then the review panels comments regarding limitations in i) the general approach and study design chosen by the industry and ii) specific methodological aspects of the different parts of the industry reports are presented as separate sections.

4.1 Summary of industry approach and study design

To fulfil the reporting obligations described under Article 6.2 of the TPD regarding comprehensive studies of priority additives, the industry conducted a literature review and laboratory studies. In short:

- **The literature review** covers toxicity of the additive itself, addictiveness, facilitation of inhalation, pyrolysis, main stream smoke chemistry (including transfer rates), and toxicity of the additive when used as a tobacco additive (testing of main stream smoke)
- **Smoke chemistry** studies analyzing the WHO list of 39 priority emissions plus tar and water in mainstream cigarette smoke using the ISO smoking regime (1). A comparative chemical analysis was performed for test cigarettes containing three levels of the additive (Low, Max, and Max-plus), and an additive-free reference cigarette.
- *In vitro* **toxicology** testing was performed using Ames test (for total particulate matter, TPM) for mutagenicity, Neutral Red Uptake in the CHO cell line (for TPM and gas vapor phase; GVP) for cytotoxicity, and the Micronucleus assay (with TPM) for genotoxicity. As for the chemical analysis, comparative testing based on these *in vitro* assays was performed for the three different test cigarettes and an additive-free control cigarette.
- **A clinical study to** assess the effect of the additives on the facilitation of inhalation and nicotine uptake. This was a controlled double-blind study using a randomized crossover incomplete block design.
- **A sensory study** to determine whether priority additives give cigarettes a characterizing flavor other than tobacco. A step-wise procedure comprising different sensory methodologies (clustering, 'In/Out' Test and CATA (Check all that applies) testing) was used in this study.

In the concluding section of the industry reports, a short summary of the literature review is presented covering chemistry and toxicity. In addition, the main findings of each of the laboratory studies are summarized. Thus, an integrated discussion of the conclusions based on the literature review and the new results from the industry laboratory studies is not included.

4.2 Limitations in approach and study design

The review panel identified several limitations in the overall approach and the applied methodology. As pointed out in Chapter 1 to 3, the review panel and the industry consortium interpreted the TPD differently, and some of the shortcomings in the industry approach that the review panel has identified are likely to stem from these differences in interpretation of the TPD. Most importantly:

- The industry reports have chosen to base their reports on both Article 6 and 7 of the TPD, and use Article 7 as an argument for a comparative testing approach. In contrast, the review panel's interpretation of the TPD is that the work of the review panel should be based on Article 6 only, and not on Article 7. Thus, the review panel does not agree that Article 7 is a valid argument for relying only on comparative testing for the chemical and toxicological analysis.

- The industry's interpretation of Article 6.2 with regard to toxicity testing is that only evidence for the additives *increased* effect size of the endpoint in question is covered, while the review panel's interpretation is that both evidence for i) the *contribution* to the endpoint studied, and ii) the *increase in effect size* of the endpoint studied. Thus, in the review panel's opinion, further data are required to fulfil the reporting obligations specified in Article 6 of the TPD. This includes data for assessing the additives contribution to toxicity, for instance new pyrolysis experiments, evaluation of the inhalation toxicity of the additives, and a toxicological evaluation of the pyrolysis products and comparative chemical analysis.

Please refer to Chapter 2 and 3 for a more extensive explanation of the ambiguity of the TPD and the differences in the industry's and review panel's interpretation.

In addition to these limitations that are likely to be due to different interpretations of the TPD, the review panel also identified other weaknesses in the approach chosen in the industry reports:

- The lack of an integrated discussion of the new results from the industry laboratory studies and the conclusions based on the literature review.
- Similarly, the results from the pyrolysis experiments (literature) and chemical analysis (literature and own data) are not used in the toxicity evaluation. Thus, an integrated discussion of chemistry and toxicity is lacking. Likewise, the chemistry data are not included in the evaluation of addictiveness.
- In the industry's assessment of cigarette smoke chemistry, the review panel does not agree with the selection of experiments performed, more specifically, the transfer rates are only assessed for a selection of additives and no new pyrolysis experiments were performed. In addition, the list of analytes included in the comparative smoke experiments was not expanded with known pyrolysis products reported to have CMR properties. These aspects are described under methodological limitations in the next section (Section 4.3).
- In the industry's assessment of toxicity, the review panel does not agree with the overall approach. Although a general toxicity assessment of the additive (based on ingestion) is included in addition to comparative experiments, there is a lack of exposure assessment, assessment of inhalation toxicity of the additive itself, the toxicity of pyrolysis products (general and inhalation toxicity) and inclusion of other endpoints than CMR properties (e.g. irritant and sensitization effects). Some of these aspects are discussed under methodological limitations in the next section (Section 4.3). See also Chapter 3 for an overview of approaches for toxicity assessment (under Section 3.3.2).
- While the studies in the industry report do not adequately assess addictiveness, the industry argues that there are no other validated or suitable methods available to address addictiveness of tobacco additives. The review panel agrees that methods such as classical intravenous self-administration in rodents would hardly be feasible to test the consequence of an additive on tobacco addictiveness. However, recent research development has led to the setup of vapor chambers that could be used to tackle the question. Furthermore, it is known that some compounds such as aldehydes, which inhibit monoamine oxidases, increase tobacco addictiveness. Therefore, increases of the quantity of any monoamine oxidase inhibitors (MAOI) following the presence of a specific additive should have been included in the report as a risk factor for addictiveness.

Prior to the deadline for providing reports based on the enhanced reporting requirements for tobacco industry, the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) proposed a guidance and a template for the drafting of these reports (2). Overall, the industry reports do not follow these available SCHEER guidelines. That is, the industry consortium has followed some of the recommendations (e.g. use of WHO list for chemical assessment instead of the outdated Hoffmann list, choice of *in vitro* tests as recommendations), while several other recommendations were dismissed without an explanation (e.g. recommended molecular level approach for chemicals

(QSAR) was not applied, no new pyrolysis studies were performed, main stream smoke chemistry and toxicology were assessed with a comparative testing approach). Some of the inconsistencies between the industry's approach and the SCHEER recommendations are described in the next section (Section 4.3). However, it should be noted that as the review panel did not base its evaluation exclusively on the SCHEER report, not all inconsistencies are outlined.

4.3 Methodological limitations

In addition to the concerns regarding the overall approach chosen in the industry reports (see previous Section 4.2), the review panel identified a range of methodological limitations in all five topics covered by the industry reports; the literature search, and the evaluation of the additives effect on chemistry, toxicity, inhalation facilitation and nicotine uptake, as well as characterizing flavors. These limitations are described in the followings sections. In addition, some of the consequences of the approach chosen by the industry are further discussed.

4.4 Literature review

In general, the industry reports present two literature reviews: one regarding the additive in general and a second one regarding its toxicological effects when used as a tobacco additive. Although the strategy displayed for the literature reviews was provided in the appendix (databases queried, review equations, keywords used, exclusion criteria, etc.), the reviews seem very partisan. For example, very few independent studies are presented. This is a source of bias and reflects a non-systematic literature search. Whether and how the quality of the studies to be included was assessed is not reported either. Finally, the reported literature does not cover all relevant areas of research. Some examples are provided in the individual additive reports.

Taken together, the members of the panel conclude that the literature reviews in the industry reports are biased and incomplete. This severely limits their usefulness for risk assessment and represents a major limitation of the industry reports. Overall, the fact that key scientific literature is missing, gives the impression of inadequacy of the full report.

4.5 Comparative testing: aspects common for chemistry and toxicity

The smoke chemistry and *in vitro* toxicology studies were performed using test cigarettes containing additives in three different application levels and/or as part of (a) mixture(s). The results were compared with those of an additive-free reference cigarette. These experiments are referred to as comparative testing. The review panel identified three methodological limitations that are common for the chemistry and toxicity comparative testing; the cigarette composition, the smoke generation method and the statistical analysis. These are addressed in the following sections.

4.5.1 Composition of cigarettes and reference products

For the comparative testing approach, special additive-free reference cigarettes have been manufactured. These reference cigarettes did not contain any of the investigated additives. In test cigarettes each studied additive was added at three different levels to cigarettes that did not contain any of the other investigated additives. Further, three 'Mix' cigarette batches were prepared. Although this approach is in general reasonable to study differences in smoke chemistry, the review panel encountered the following shortcomings that affected both the chemical and toxicological comparative testing:

- When the humectants glycerol and propylene glycol were investigated as priority additives, the

additive-free reference cigarettes and most test cigarettes did not contain either substance. However, humectants are technically necessary and greatly influence combustion conditions and therefore the emissions of most compounds. Thus, these cigarettes can be regarded as faulty references. The industry should instead have compared cigarettes with different humectants to find the option with the least concern, both with respect to chemistry and toxicity. This limits the usefulness of the whole data set for glycerol and propylene glycol.

- The identity of all the compounds and their exact quantities added in the reference cigarettes and other tested cigarettes was missing, but needed. This information was later provided by the industry upon request (also see Annex IV).
- Next to the identification of all compounds, it is necessary to know the absolute quantities of nicotine and sugars present (amounts lost during curing of the leaves, complemented for this loss and additionally added) in the 3R4F reference cigarette and other tested cigarettes. This information is, for instance, necessary for the evaluation of addictiveness because sugars give rise to aldehydes, which are potent monoamine oxidases inhibitors which themselves increase addictiveness of tobacco products.
- Another problem is that the production of test cigarettes is a monopoly of tobacco industry. The review panel proposes that these type of products should be made available for independent research.

4.5.2 Smoke generation

For smoke composition in the comparative experiments reported by the industry, smoke was generated using the ISO method, which is a standard that has been adopted many years ago and was developed with involvement of the tobacco industry. Over the recent years, discussions within the WHO and other research institutes have focused on the importance of independent methods, how this method relates to how smokers use the cigarette in 'real life' and whether the mainstream smoke acquired by the ISO method is comparable to what smokers are exposed to (3). The current consensus is that the ISO method leads to underestimated amounts of mainstream smoke (MSS) products. This is due to several reasons. Firstly, the low puff frequency and puff volume leads to smaller amounts of smoke ´inhaled´ from one cigarette. In addition, the low intensity might result in lower temperatures leading to less MSS products. Importantly, when using the ISO method, ventilation holes in cigarette filters allow influx of clean air, diluting the smoke, whereas smokers tend to cover these holes with their lips and fingers, and/or inhale larger or more frequent puffs to compensate for diluted smoke. Due to more intense smoking and closing of the ventilation holes, greater quantities of harmful substances end up in the smoke (4). Another smoke generation method developed by Health Canada and validated and recommended by WHO represents more intense puffing and combustion conditions and also takes into account the covering of ventilation holes (3). This method is known to produce higher emission levels, which are closer to human exposure (although still not perfectly representative). In the opinion of the review panel, data resulting from both ISO and WHO Intense smoking regimes would be needed for a proper risk analysis.

4.5.3 Statistical approach

The same statistical approach was used for the analysis of the cigarette smoke composition and the smoke toxicity analysis. A statistical equivalence approach was applied using variations of the (noncommercial) 3R4F monitor cigarette as a benchmark to determine whether variations of the mean values of both test and control cigarettes show significant differences. Accordingly, differences between test cigarettes (low, max, max plus) and control (reference) were not considered as relevant, when smaller than the variability of the 3R4F monitor cigarette. This approach is explained by Belushkin et al., (2015) in detail (a paper funded by the tobacco industry) (5).

In the benchmark approach, the industry reports use a 99.7% confidence interval, which reflects allowing a 0.3% chance of false positives (detecting a significant difference when there is not an actual difference). In other scientific literature, 95% confidence intervals are typically used. To specify confidence in the variability of the 3R4F monitor cigarette to 99% leads to a 1.5-fold wider range (3 x SD) as compared with a 95% confidence requirement (2 x SD). Consequently, application of the 99% confidence benchmark allows larger differences between test and control cigarettes to fall into this range and subsequently to be regarded as non-relevant. This may lead to false negative results (not detecting a significant difference when in reality there is a difference), and is a major methodological limitation.

For the analysis of the cigarette smoke composition and the smoke toxicity analysis, data are compared to a historical variation over a longer period (the industry report says *"time period: 2013 – 2015"),* based on either the ISO (chemical composition) or Canadian Intense (toxicity analysis) regime. The use of historical data variation will lead to an overestimation of the variation that can be expected within the study itself. Accordingly, any deviations from the reference product are less likely to be statistically significant and this may lead to false negative results. This, too, is a major methodological limitation.

Note that this benchmark approach, with a 99.7% confidence interval and comparison with historical data is not a common statistical approach in toxicological or chemical assessments. For instance, for environmental exposures like particulate matter, a common approach is to use a 95% confidence interval and use the control condition from the same experiment as a reference (6).

The evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. If any difference in chemistry or toxicity exceeded the 3 x SD variability in the 3R4F monitor cigarette, the mean concentrations for each of the three test cigarettes (Low, Max, Max-plus) and the additive-free reference were compared using analysis of variance (ANOVA) with significance evaluated at $p = 0.05$. If the ANOVA showed a statistically significant effect, mean analyte concentrations among the (Low, Max, Max-plus) test cigarettes were compared to the additive-free reference cigarette using the Dunnett's test (with a family wise error rate of $\alpha = 0.1$). This was followed by a linear trend analysis for a consistent additive-concentration related decrease or increase. Only if statistical significances were found for all three of these tests (as follow-up to exceeding the 3R4F variation), differences were reported as significant and meaningful. Because the overall statistical evaluation by the industry involved several additional analyses on the data, reporting only findings that passed all of the individual tests as significant increases, the chance of false negative results due to an additive (falsely) failing to meet the significance threshold in one or more of the tests.

Taken together, the three aspects of the statistical analysis described above (application of a 99.7% confidence interval, comparison with historical reference data and require results to pass several tests), all increase the chance of false negative results. Thus, the industry's statistical approach appears to be in favor of discovering null findings.

4.6 Chemical assessment

The review panel does not agree with the selection of experiments performed and the analyte list used for the comparative experiments. In addition, the data quality was insufficient for one series of experiments presented in the industry reports. These methodological aspects are discussed below, while the methodological aspects that are common for chemical and toxicological comparative testing were described in the previous section (Section 4.5).

4.6.1 Transfer experiments

The criteria for selecting additives to include in transfer experiments are unclear. Transfer into mainstream smoke was generally not determined in industry experiments for compounds described to have a *"complex chemical composition and non-volatile nature"*. However, for cocoa (which is also complex and non-volatile) the transfer rate of theobromine was determined since this is considered to be the biochemically active compound of cocoa (in addition to caffeine which is present in much lower levels). Thus, the selection of priority additives for which the transfer into mainstream smoke was determined appears to be somewhat random. Assessment of the transfer of active compounds of other complex and non-volatile additives should have been included in the industry reports as well.

4.6.2 Pyrolysis products

The SCHEER report suggested that new pyrolysis experiments should be performed by the industry. However, the industry argues against use of data from pyrolysis experiments based on their limited relevance for predicting the presence and levels of pyrolysis products in mainstream cigarette smoke. They state that pyrolysis can only approximate combustion of a burning cigarette in the case of non-volatile additives and might not realistically reflect thermic decomposition during smoking. They also state that there is no correlation between results of pyrolysis experiments and smoke chemistry (other than for volatile compounds that have been added in small amounts), and that *'to assess how tobacco additives influence the quantitative levels of toxic substances in whole smoke, i.e. mainstream smoke, the pyrolysis of additives has been deemed not suitable as an assessment criterion (Hahn and Schaub (2010))'(7).* The review panel agrees in that pyrolysis experiments cannot predict main stream smoke chemistry, but the consensus in the panel is that these experiments still provide useful information about compounds that are found in smoke.

The industry's evaluation of pyrolysis experiments is mainly based on two publications from 2005 by Baker and Bishop (8, 9). In these pyrolysis experiments, besides the five most abundant pyrolysis products, the inclusion of hazardous components was based on the Hoffmann list, developed in the 1990s (10-12). However, a more updated list of compounds, like the one developed by Talhout and colleagues (2011) (13), is required since it also includes compounds that affect respiratory and cardiovascular endpoints in addition to carcinogens.

In the review panels opinion, new pyrolysis experiments should have been performed to identify pyrolysis products based on an updated list of compounds, with a subsequent toxicological evaluation of the pyrolysis products. This is in line with previous recommendations. For instance, although the paper by Hahn and Schaub (2010) cited by the industry states that that *'pyrolysis of additives itself is not sufficient as an assessment criterion',* the authors also suggest a four step model for toxicological assessment of tobacco additives that includes pyrolysis experiments and toxicological evaluation of pyrolysis products as the second step (7). The SCHEER report also recommends toxicological evaluation of the pyrolysis products and use of an updated list of compounds for identification of toxicants (2).

The review panel did an independent evaluation of the pyrolysis products presented by the industry based on the two Baker and Bishop (2005) publications (8, 9). This evaluation is presented in Annex III, and was used in the evaluation of the individual additives presented in Chapter 6.

4.6.3 List of analytes

In accordance with the SCHEER recommendations, the list of analytes used for the comparative chemical analyses was based on the WHO list of 39 priority emissions (plus TPM, tar, and water). However, previously identified pyrolysis products of each additive based on the literature review were not added to the analyte list. This is a major shortcoming, since several of these previously

identified pyrolysis products have been classified as compounds with CMR properties (e.g. furfural), as described in Annex III. However, assessment of pyrolysis products in the mainstream smoke in comparative experiments is required for a toxicity evaluation of the contribution of the additive to the toxicity of a tobacco product (as outlined in Chapter 3).

4.6.4 Data quality of chemical analysis

The quality of provided data on mainstream smoke composition varied throughout the reports, limiting their usefulness. The comparative mainstream smoke experiments presented in the industry reports were performed in two separate sets. The first set consisting of carob bean, cocoa, fenugreek, fig, glycerol, licorice and menthol seems to have been performed under relatively acceptable conditions, resulting in standard deviations of approximately 10% (Figure 4.1a). The second set, consisting of geraniol, guaiacol, guar gum, maltol, propylene glycol and sorbitol, resulted in much higher standard deviations (Figure 4.1b). The example provided in the figure, for a selection of carbonyls, demonstrates that the variability in both reference and test cigarettes was much higher for geraniol than for carob bean. In fact, for the same additive-free reference cigarette that was measured in both sets, the standard deviations were reported to range from 7-14% for set 1, and 33-55% for set 2. This implies inconsistencies in the laboratory procedures. Interestingly, previous studies from both industry and independent researchers achieved low standard deviations of approximately 10% in levels of emitted carbonyls (14-18).

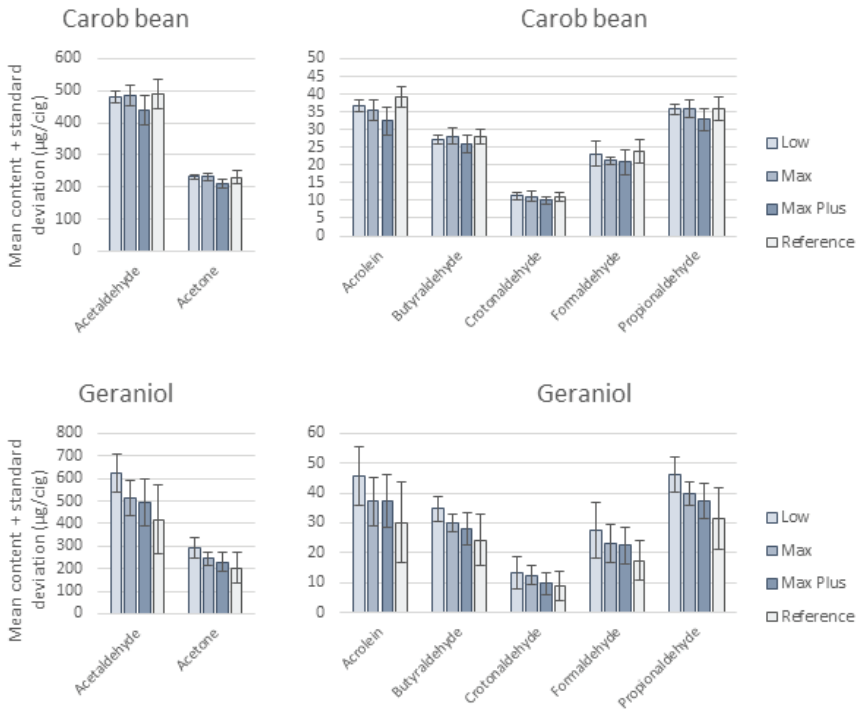

Figure 4.1: Example of acceptable and high standard deviations in reference cigarettes. Mean and standard deviation of carbonyl emission levels after application of a) application of low (0.2%), max (0.4%), and max plus (0.6%) levels of carob bean and corresponding additive-free reference cigarette resulting in acceptable standard deviations or b) low (0.015%), max (0.03%), and max plus (0.045%) levels of geraniol and corresponding additive-free reference cigarette resulting in high standard deviations. The figure is based on data from the industry report.

Overall, the data resulting from the second experimental series showed large variability and the data do not appear suitable for a regular statistical analysis. To allow for extraction of useful information from the data provided by the industry in spite of the poor data quality, an independent evaluation of the carbonyl data was performed by the review panel. The applied stepwise criteria are described in Chapter 3, Section 3.3. The results of the independent evaluation, presented in Annex II, were used in the evaluation of the individual additives presented in Chapter 6.

4.7 Toxicological assessment

The review panel does not agree with the overall approach of the industry for the evaluation of toxicity. An overview of a preferred approach for toxicity assessment of tobacco additives is provided in Chapter 3, this includes assessment of A) the general toxicity of the additive (based on ingestion), B) inhalation toxicity of the additive itself, C) the toxicity of pyrolysis products (general and inhalation toxicity) and D) comparative testing. In general, the industry reports focused on the toxicity of the additive per se and *in vitro* comparative toxicity testing (points A and D), while no data were presented for inhalation toxicity of the additive itself or its pyrolysis products (points B and C). Some consequences of the industry's selective evaluation of toxicity are discussed in this section, while the methodological aspects that are common for chemical and toxicological comparative testing were described in Section 4.5. In addition, the main limitations associated with the comparative toxicity testing are addressed below.

4.7.1 Pyrolysis product toxicity

A toxicological assessment of identified pyrolysis products was not included in the industry reports. However, in the independent evaluation of the pyrolysis products performed by the review panel (Annex III), several pyrolysis products with CMR properties were identified. Thus, these pyrolysis products could contribute to the toxicity of the cigarettes (see Chapter 3 for a discussion of how this relates to the interpretation of the TPD). Note that verification of the presence of the pyrolysis products in mainstream smoke is required for a meaningful risk assessment of pyrolysis products. Nevertheless, a more complete assessment of the toxicity of the already identified pyrolysis products is warranted, as well as identification and evaluation of novel pyrolysis products based on a more updated analyte lists than the Hoffmann list (see also section 4.6.2).

4.7.2 Selection of assays and endpoints

For the *in vitro* assays used in the comparative testing, the main parameters allowing the review panel to judge the adequacy of the model with the endpoint tested are not discussed. In an appendix (B) of an Annex (A), the CHO cell line (ovary cells) is mentioned for Neutral Red Uptake (NRU) and *in vitro* Micro Nucleus (ivMN) tests. This model is used based on the following justification: *"The selection of the test system is based on the requirements of the Health Canada official test method T-502, Second Edition, 2004-11-01. Labstat has validated this test system during the method development of the NRU assay."* In the main text under literature review, the V79 Chinese hamster cell line (lung cells) is mentioned. The differences of these two models, with regard to relevance, tissue of origin and sensitivity, are not discussed. Neither are the consistencies or discrepancies in results obtained by these different models. Recent work published by the Tobacco industry have discussed this issue (19). In general, an *in vitro* model is appropriate only if it has been proven as relevant to inform on a specific question, including in particular the reliability of extrapolation of the data to the human situation. In the industry reports, the differences in the doses applied, positive controls etc. are not discussed either, rendering the provided *in vitro* studies difficult to evaluate for the review panel.

The presented toxicity data focus solely on CMR properties. Although this is reasonable since carcinogenic effects are the best-characterized adverse effects of smoking cigarettes, other adverse effects have also been reported to be associated with cigarette smoking and can interfere with or facilitate CMR properties. In particular, irritation is a known promotor of carcinogenicity. Thus, other relevant adverse effects induced by additives, such as irritation, sensitization and cardiovascular effects should also have been included in the reports.

4.7.3 Comparative toxicity testing

The toxicity evaluation in the industry reports includes a general toxicity assessment of the additive (based on ingestion) and evaluation of comparative experiments (based on literature and their own study). As summarized in Section 4.2 above, the industry uses Article 7 to argue for relying mainly on comparative testing for toxicological analysis, while the review panel has a different interpretation of the TPD (see Chapter 2, 3 and above).

The main limitation of the comparative testing approach is that these studies lack discriminative power due to the high background toxicity of tobacco products (SCHEER 2016) (2). More specifically; *"comparative toxicity testing strategies, where differences in the effect of the tobacco product with and without the additive are evaluated, are not considered suitable to address the properties outlined in the Terms of Reference with the currently available methodology. Indeed, at present, these studies lack discriminative power due to the high toxicity of tobacco products themselves and their results cannot be generalized to all products and brands, having a different composition with respect to tobacco type, blend and additives. Here, the effects of the pure additive, and its pyrolysis products, are considered in order to evaluate their contribution to tobacco product toxicity."*

Another limitation of the comparative toxicity testing performed by the industry is that only *in vitro* tests were included. Although these *in vitro* tests were in line with the recommendations from SCHEER (2), they are of limited value in the assessment of CMR properties as *in vivo* testing is currently unavoidable to establish certain aspects of CMR properties, in particular for non-genotoxic carcinogens. However, suitable comparative testing cannot be conducted in a manner allowing scientifically powerful studies with ethically acceptable design (i.e. too many animals would be necessary for *in vivo* comparative testing). Based on the scientific literature and our shared knowledge as a review panel we do not have a proposed scientific methodology for fulfilling our request for evidence for the increased effect size of the additive on the endpoint studied requested by the TPD. Thus, a revision of the TPD may be required, as pointed out in Chapter 3. In such a revision, the possible use of some assessment methodologies (e.g. Mode of Action and Adverse Outcome Pathway), which do not necessarily need new animal studies, should be considered.

4.7.4 Lack of integrated and interdisciplinary evaluation

The industry reports lack a comprehensive and interdisciplinary approach in the evaluation of the data presented for chemistry and toxicity. The chemical analysis data are not summarized to conclude regarding the additives most important chemical characteristics. Then, the data from the chemistry section are not applied in the toxicological evaluation. For instance, the identified pyrolysis/combustion products are not evaluated in terms of inhalation toxicity. Similarly, the aldehydes which inhibit monoamine oxidases, and thereby may increase tobacco addictiveness (see below), were not assessed in terms of possible implications for addictiveness although this was pointed out in the SCHEER report (2).

The application levels and transfer rates of additives are presented in most reports, but not applied in the toxicological evaluation. In fact, there is no exposure assessment included for the priority additives at all in the industry reports, although this is required according to standard risk assessment procedures (20). Another important factor that was not taken into consideration in the industry reports is that mixtures of compounds may produce additive and synergistic toxicity at concentrations where the individual components are of lower concern.

4.8 Inhalation facilitation, nicotine uptake, and addictiveness

The industry provided a clinical study measuring plasma nicotine pharmacokinetics as a measure of nicotine uptake, and smoker puffing behavior as a measure of cigarette smoke inhalation. In this study, 10 out of the 15 priority additives, are studied as a single additives. Three additives (geraniol,

guar gum, and maltol) are only studied in a mixture, while the two remaining additives (diacetyl and titanium dioxide) are not included in this study. Compared to the toxicity and chemistry section, this part of the report was considerably smaller in size. It should be noted that an abundance of information about the clinical study was presented in an unclear fashion, which significantly hindered the assessment of the applied methodology.

The clinical study also had several methodological shortcomings rendering these data incomplete and difficult to use for risk assessment. More specifically:

- Endpoints in the clinical study were measured after single use of one test or reference cigarette by the participants. Participants visited the test clinic on eight occasions, during each of which they smoked one test (each time for a different additive) or reference cigarette. During the study period, participants were allowed to continue smoking their own brand of cigarettes. However, as addiction develops as a consequence of neuroadaptation after repeated exposure to addictive substances a measurement after single use of a cigarette is not representative for such effects.
- No additional endpoints for dependence potential are assessed, including relevant clinical endpoints like craving, dependence, and withdrawal symptoms. Although the current test design is not suitable for assessing such endpoints, these could have been addressed with a different study design where smokers switch to the tested cigarettes for a prolonged duration.
- Measurements of plasma nicotine pharmacokinetics (PK) were conducted before, during and after smoking the first cigarette in the morning after a night of abstinence. It is well known that sustained abstinence overnight increases nicotine craving and withdrawal. This may have led to more intense smoking behavior by the participants, thereby minimizing differences in PK parameters between test and reference cigarettes.
- Measurements of smoking behavior parameters were performed when participants were smoking a second cigarette, 4 hours after the first. As the level of dependence of participants ranged from very low to very high (i.e. Fagerström scores 1-9), 4 hours between cigarettes may have caused none to very strong craving and withdrawal, possibly causing considerable variations in the measurement.
- As the study participants were all addicted smokers, results are not generalizable to the development of addiction in smoking naïve, and possibly more sensitive, people. The review panel acknowledges that research on nicotine naïve human subjects is unethical, but animal and *in vitro* studies could inform on this topic.
- A statistical assessment was only performed for PK parameters, whereas for the smoking behavior parameter analysis, only descriptive statistics were provided. The rationale from the industry for this was that *´the statistical analyses of PK parameters revealed no significant differences between the PK parameters for the corresponding test and reference products´*. However, not finding significant differences in one test does not exclude the possibility of significant differences the other, and thus a formal statistical testing would be required in order to evaluate the smoking behavior data.
- Three additives are only studied in a 'Mix' test cigarettes and not individually. A suitable rationale for this approach is not provided in the industry reports.
- Generally, the industry does not rely enough on independent literature when available, although several additives have been implicated in addiction to tobacco and derived products in independent studies.

4.9 Characterizing flavors

For assessment of characterizing flavor properties, the industry performed a sensory testing using a consumer panel. This assessment was performed for only eight out of 14 additives, and did not include glycerol, guar gum, maltol, propylene glycol, sorbitol, and titanium dioxide. Two members of the review panel are Pr. Efthimios Zervas, Chair of the Independent Advisor Panel (IAP) on characterizing tobacco flavors and Dr. Constantin Vardavas, member of Technical Group (TG)

assisting IAP. Moreover, the review panel consulted an expert from the TG, Dr. Bill Simpson. Based on their shared expertise, several methodological flaws in the industry´s assessment were identified, as summarized in the following sections.

On the basis of the evidence presented in the industry reports it is not possible to conclude whether the tested additives can be responsible for introducing a characterizing flavor or odor to tobacco products. This is primarily due to the many uncertainties relating to the sensory panel composition, the methodology applied in the evaluation of characterizing flavors and the interpretation of data.

4.9.1 General limitations

- One of the tasks of the Independent Advisory Panel is to specify and, as appropriate, update the methodology for the technical assessment of test products (Commission Implementing Decision 2016/786). As this methodology was not available the time of the industry tests, the previously established recommendations for the identification of characterizing flavors in tobacco products comes from the Health Effects Tobacco Composition (HETOC) consortium. These recommendations were however not followed by the industry, nor another international standard, and there is no explanation provided of the rationale for this.
- It is important to note that some additives are pure compounds and/or mixtures that can be altered during several stages: the production process of the extract or powder, the processing phase of the extract, the cigarette manufacturing phase, storage and during smoking. For this reason, there are some time-dependent changes that can affect both flavor character and intensity. Consequently, vital information is missing from the Consortium Report that could be a determining factor in whether or not the additive would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which the additive was incorporated in the tobacco product, as well the quantity of the remaining additive during the sensory tests. If the quantity of the additive remaining in the test cigarettes is lower than the quantity originally added, the flavor impact of the additive will be underestimated.
- In general, it is of paramount importance that sensory tests conducted to determine the flavor impact of additives are conducted under conditions that are not likely to give rise to biased responses. Minor choices in relation to test design variables can have a big effect on test outcomes and could result in the impact of product flavor being grossly underestimated. The choice of statistical tests and criteria to decide on whether or not a product possesses a characterizing flavor is also of great importance. The studies described in the industry report have shortcoming in this respect (conditions, test design and statistical tests used) and are likely to give biased results.

4.9.2 Limitations regarding the consumer panel

- In the industry study, untrained consumers describe flavors, while this should preferably be done by trained experts.
- Moreover, the number of participants, for both experts and consumers tests, must be of large size. Although sample sizes in consumer studies vary based on e.g. consumer characteristics, purpose of the test, timeframe and cost, the commonly required minimum sample size consists of several dozens of participants, at least 40-100 consumers. (21-23) The panel of 10 consumers used in step 2 of the industry assessment, is therefore too small to produce reliable results. Similarly, the trained experts used in the panel are only three, where there should have been at least 10 (24).
- Furthermore, a strong argument can be made that the primary target consumer for the tobacco industry in the use of characterizing flavors in tobacco products is i) young, and ii) a non-smoker. Studies in which assessors of a wide diversity of age are recruited, and which restrict participation to smokers only, will lead to an underestimation of the impact of a substance on

the flavor of tobacco, since these individuals will be less sensitive to the characterizing odor than many other individuals (as their sensory capability is modified). The reported studies used assessors who were likely to have lower sensitivity to the odors of priority additives in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits.

- The screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor. Screening was based on beverages (different brands of cola, water taste, sweetness ranking) and odor recognition of mostly easy and irrelevant smells (e.g. vinegar, lemon, grass)

4.9.3 Limitations regarding sensory test design

- The sensory analysis consists of a number of sequential steps (identification of reference products; screening using an In/Out test; sensory analysis using CATA (Check All That Applies) testing; Analysis of the CATA data). As each step sets limits for the next steps in which products and parameters are being evaluated, this significantly increases the chance of false negative results.
- There is an unmotivated choice of cutoffs for the In/Out tests. Only products for which 6 or more of the 10 consumers identify the test product as 'Out' of the reference product range are subsequently tested for characterizing flavors (CATA). This cutoff criterion of 6 out of 10 consumers is i) arbitrary and ii) high; therefore the selected products are per definition positive controls due to previous steps and biased reference sample.
- The reference tobacco applied in the sensory analysis is not appropriate. This includes the existence of menthol in the reference cigarette, while they assume that reference products (which are representative of the different brand types of the European market) do not have a characterizing flavor. This has yet to be proven.

4.10 Attractiveness

Even in the case of an absence of characterizing flavors, the addition of priority additives with (even light) specific flavors can increase the attractiveness of cigarettes and RYO. It is also known that by the heating of sugars, secondary products are generated which have an attractive smell and taste, thus increasing product appeal and promoting smoke inhalation and possibly influencing addictiveness by increasing the level of nicotine exposure. Moreover, some additives decrease the harshness and increase the smoothness of the smoke, while others may be used to dilate the airways allowing the smoke an easier and deeper passage into the lungs. Certain additives yield a full and white smoke and other additives reduce the lingering odor of the smoke to increase acceptability of smoking to smokers and to people around. Generally, additives increasing attractiveness may lead to brand preference or an increased consumption of tobacco products (25, 26). The industry reports do not contain any information about the potential increase of attractiveness of cigarettes and RYO due the addition of priority additives. Even though this was not specifically required by the TPD, the review panel considers this a significant shortcoming.

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Chapter 5. Additive reports

Disclaimer: This Chapter provides reports on the evaluation of industry studies of individual additives. It should be noted that the conclusions presented are based on the data reported in the industry reports as well as some additional data from independent sources that was consulted by the reviewers. A comprehensive review of independent literature was not requested from the review panel and not feasible within the allocated resources. In addition, conclusions only apply to the currently tested application levels and cannot be extended to higher levels.

Sentences written in italics are the same in each report.

5.1 Report of Carob bean

5.1.1 Abstract

Carob bean is widely used in the food industry where powdered seeds (carob bean gum) are applied as thickener, stabilizer and flavor. Carob bean extract is, predominantly as flavor or flavor modifier, used in tobacco products in application levels up to 0.4% of tobacco weight. In the industry report three levels are tested 0.2% (Low) 0.4% (Max) and 0.6% (Max-plus). Carob bean is a complex mixture of mainly non-volatile compounds. A transfer of these compounds into mainstream smoke is unknown but unlikely and has not been studied. The pyrolysis product furfural (1.5 %, Carc. 2) has CMR properties. Industry concluded in their report that application of carob bean did not result in significant effects regarding smoke chemistry, toxicity, addictiveness, inhalation facilitation or characterizing flavor. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. More data is needed to determine to which extent carob bean adds to providing a more appealing taste and decreasing smoke harshness and thus facilitating

inhalation. The main concerns of the review panel with regards to using carob bean as an additive in cigarettes and roll your own tobacco are the carcinogenicity of pyrolysis products (e.g. furfural), the possible inhalation facilitation, and its flavoring properties that enhance attractiveness.

5.1.2 Background

Carob bean is a natural complex mixture of different chemical compounds, mostly carbohydrates/ sugars. Together, these compounds are used as a flavoring material in commercial cigarettes. It imparts a sweet and nutty taste that enriches the smoke flavor. Each of the compounds in carob bean can separately, or interactively together, have addictive, harmful or toxic characteristics. Carob bean has many forms such as extracts and gum, which are frequently added to tobacco products at relatively high amounts, up to 0.4%.

Several concerns were identified for carob bean in tobacco products in the PITOC factsheet (1) and the SCENIHR report (2).

Regarding toxicity: The effects of carob bean inhalation through smoking have not been studied. The risk associated with the generation of combustion products upon carob bean pyrolysis has not been thoroughly studied and therefore, an adequate risk assessment for carob bean or its pyrolysis products by inhalation is currently not available. However, the pyrolysis of sugars, its major components, is well reported and gives rise to a complex mixture of toxic, carcinogenic and mutagenic compounds.

Regarding addictiveness: Carob bean functions as an antitussive active ingredient and can reduce the harshness of the smoke, which may facilitate inhalation. Constituents, metabolites and pyrolysis products from carob bean are considered to inhibit monoaminoxidase (MAO) based on existing literature data (3). MAO inhibition is known to enhance addictiveness.

Regarding characterizing flavor: Carob bean can alter the sensory properties of cigarette smoke, by providing a more appealing taste and decreasing its harshness.

5.1.3 Literature review

The industry report provides two literature overviews for carob bean, one regarding carob bean in general and a second one regarding carob bean when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4).

Carob bean's antitussive property, which may facilitate inhalation, is not evaluated in the report. During combustion, sugars are also known to produce aldehydes that can enhance the addictive potential of nicotine. These sugars-related issues, yet central and well documented in literature, are not sufficiently addressed in the report.

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.1.4 Chemistry and Pyrolysis products

The report provides a brief description of the additive, as well as lot properties. Carob bean extract is used by the tobacco industry without further specifications (i.e. purity). Submitted data cover

information on the manufacturer and compliance with applicable EU food flavoring legislation and manufacture of the test cigarettes.

Application levels: The main constituents of carob bean extract are water (28%) and sugars (68%), especially glucose and fructose. Carob bean levels applied in test cigarettes varied between 0.2% and 0.6% (Low 0.2%; Max, 0.4%; Max-plus 0.6%). These levels are in line with the application levels currently used in commercial cigarettes, reported to be maximum 0.4%, although achieved concentrations after application of carob bean have not been tested by the industry ("Technically not possible to detect achieved amount").

Transfer of carob bean into mainstream smoke: Based on the non-volatile properties of its major constituents, the industry concludes that transfers of intact molecules into mainstream smoke are very unlikely. Carob bean is a mixture of several compounds that have been shown to undergo pyrolysis. The industry report claims that there are no studies in the literature and for the transfer of carob bean constituents into mainstream smoke, and no new tests were performed.

Pyrolysis experiments: The industry report takes reference to published studies on pyrolysis of carob bean extract, most importantly Baker and Bishop (4). Major pyrolysis products of carob been powder are acetic acid (23.5 %), hydroxymethylfurfural (13.3%), furfural (8.1%), benzoic acid (7.3%) and acetol (6.3%). Further, it is acknowledged in the industry report that experimental pyrolysis of non-volatile additives can only approximate combustion of a burning cigarette and, for this reason, pyrolysis results are not predictive for smoke chemistry. No new pyrolysis experiments were conducted.

Chemical analysis of mainstream smoke: For comparative testing of main stream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.2 - 0.6 %).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, are described in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results . Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.

In the industry experiments, the comparative testing approach showed some differences between the test cigarettes and the additive free reference product. The difference in levels of NNN, NNK and water exceeded the variability of the 3R4F monitor cigarette at the low application level only. However, the statistical analysis performed did not result in any overall statistically significant increase or decrease of any of the tested chemical compounds. In the literature, addition of carob bean has been reported to increase emissions of particulate matter and of several compounds (such as formaldehyde, acetaldehyde and other carbonyls, NH3, PAH, heavy metals, etc.) compared to reference cigarettes (5-9).

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase

tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). No increase of carbonyls was found in the test performed by the industry at levels tested.

Evaluation by the review panel: The submitted report on carob bean extract examines the transfer, pyrolysis, as well as the levels of harmful and potentially harmful constituents in cigarette smoke. However, no new pyrolysis experiments were performed and there are limitations in the comparative testing approach. According to the industry report, carob bean extract is applied up to 0.6% (Max-plus) in tobacco products. It is a complex mixture of compounds that undergo pyrolysis. The industry concluded that no statistically significant and consistent additive-level related increases or decreases were recorded for any smoke constituent in comparative experiments. Increases in emissions were not additive-level related. Although, there were limitations in the approach and methodology, the members of the review panel conclude that no additive-level related effects on smoke chemistry after addition of carob bean extract at tested levels were found. Thus, an effect on smoke chemistry after addition of carob bean at tested levels is unlikely, but cannot be ruled out. However, as some known pyrolysis products were not included in the chemical comparative analysis, further smoke analyses should have been provided.

5.1.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – PARTLY ASSESSSED

As discussed in the Chapter 4, evaluation of the oral toxicity of an additive has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relative extensive evaluation of the toxicity of carob bean when ingested. Nevertheless, the literature search is biased and data are missing for *in silico* tests and chronic toxicity on non-rodent species. The report provided by the industry is almost exclusively based on the opinion of the EFSA ANS panel (10) and concludes that ingestion of the food additive carob bean (E 410) has no carcinogenic, mutagenic/genotoxic or reprotoxic properties. The EFSA opinion concludes to a very low acute oral toxicity potential. However, it also highlights the existence of several allergic reactions and recognized the possible potential allergenicity of carob bean gum that is not reported in the report provided by industry. Nevertheless, exposure from the diet is completely different from exposure by inhalation as carob bean gum is extensively degraded by the intestinal microflora. Therefore, it is not relevant to use the assessment that was conducted for its use as a food additive to evaluate the toxicity of the same compound when included in tobacco products. In addition, it is not known whether the composition of carob bean extract used as an additive in tobacco product is similar or not to the one used as a food additive (E 410) for which physicochemical characteristics are specified in the European regulation (231/2012 EC) (11).

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

The inhalation toxicity of carob bean is not evaluated in the industry report. Also, information regarding metabolite formation and their possible toxicity is lacking. Since carob bean is not a volatile compound, transfer in its unburnt form to mainstream smoke is unlikely. However, some constituents of carob bean and mostly its pyrolysis products may be present in the smoke. Thus, in the industry report, an initial step in assessment of the inhalation toxicity of carob bean itself should have been an identification of carob bean derived compounds transferred in smoke. The industry has not provided the transfer rate into mainstream smoke of any components, and did not evaluate the toxicity of any of them.

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The pyrolysis products identified in the report were not evaluated in terms of oral or inhalation toxicity. Moreover, the identification of pyrolysis products was based on literature applying the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of carob bean were not determined. The review panel performed an independent toxicological assessment of the pyrolysis products provided by the industry (see Annex III – "Pyrolysis product table"). One example of toxicologically relevant information that is not provided in the report is that furfural, which was identified as a pyrolysis product in the report, is classified as a CMR carcinogen cat. 2 under the EC Regulation No 1272/2008 (12). Therefore, it is likely to contribute to the toxicity of the tobacco product. Further, acetic acid is classified as skin corrosive and a mutagenic effect of pyruvaldehyde has been suggested, but evidence for this in human studies was not conclusive (IARC 3 classification, see Annex III).

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

For comparative testing, the report includes both a literature review and new studies. Based on the available comparative *in vitro* experiments, that did not identify significant effects, the industry report concludes that at inclusion of 0.2 – 0.6% of carob bean in tobacco does not increase the CMR properties of the mainstream smoke.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 0.2-0.6% carob bean does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, the review panel concludes that the provided assessment is not sufficient to reach a conclusion regarding the toxicity of carob bean when used as a tobacco additive. There were several methodological limitations in the comparative testing approach. Furthermore, only CMR properties were considered and no data were presented on the inhalation toxicity of carob bean and of its pyrolysis products. For instance, the pyrolysis product furfural is a classified carcinogen, and increase of furfural levels in mainstream smoke was not assessed. Thus, a contribution to CMR properties cannot be ruled out.

5.1.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: Although there is a lack of literature on the addictive effect of carob bean, its main constituents, carbohydrates/sugars, are well identified and are subject of many publications. Because they generate acetaldehyde in tobacco smoke, Talhout *et al.* (3) stated in their study that : "it has been speculated that acetaldehyde reacts with biogenic amines to condensation products that inhibit monoamine oxidase, an enzyme that degrades biogenic amines, like dopamine and noradrenaline" and act synergistically with nicotine. Fowler *et al.* (13) also showed in their study that: "smokers have reduced brain MAO A relative to nonsmokers" and "MAO B was inhibited by about 40% in smokers relative to nonsmokers and former smokers". Other studies (14, 15) have shown that chronic treatment with MAO inhibitors enhances the reinforcing effects as well as the motivational properties of nicotine in rats. Thus, carob bean could participate, in reinforcing the addictive effect of nicotine to some extent that still needs to be determined. Nonetheless, the industry report states that there is no evidence in the literature to suggest an addictive effect of carob bean.

Inhalation facilitation: Decrease of smoke pH leads smokers to "increase their smoking frequency and inhale the smoke more deeply to enable a higher absorption of nicotine in the airways", as well as decreases the harshness and irritation of the smoke encouraging them "to develop a smoking habit" (3). The industry report indicates that pyrolysis of carob bean leads to the formation of acids. Therefore, addition of carob bean can potentially decrease smoke pH and could then reduce harshness of smoke and facilitate inhalation. The industry report also points out that there is no data indicating that carob bean can increase the pH of smoke, whereas nothing is mentioned about the carob bean's ability to decrease the pH of smoke.

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of carob bean, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added carob bean to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of carob bean on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests.* In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from carob bean. Specifically, none of the reported studies did asses the capacity of metabolites and pyrolysis products of carob bean on monoamine oxidase inhibition. There were also no studies reported assessing the effects of carob bean on nicotine bio-availability, and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores.

Evaluation by the review panel: The provided data show no effect of adding carob bean on inhalation facilitation and nicotine uptake, but these data are limited and do not sufficiently address the previously identified concerns of MAO inhibition and lowered pH of smoke. In the independent re-evaluation of the chemical analysis of mainstream smoke data reported in the industry report (see Section 4 of this Chapter and Annex II), no increase in carbonyl emissions was found at the tested levels. Other compounds that might be relevant for inhalation facilitation or nicotine delivery were not assessed in the industry report. The fact that independent data show that the main constituents of carob bean may enhance the reinforcing effects of nicotine through MAO inhibition and reduce the harshness of smoke by decreasing its pH has not been adequately addressed. Altogether, there is insufficient evidence to rule out an influence on addictiveness or inhalation facilitation due to application of carob bean as an additive in cigarettes and RYO tobacco.

5.1.7 Characterizing flavor

Although carbo bean is a known flavoring agent, used in concentrations of only some ppm as flavor/taste modifier in food, against 0.4% in tobacco products. It is not possible to conclude whether the additive carbo bean can be responsible for introducing a characterizing flavor or odor to tobacco products, on the basis of the evidence presented in the industry report. This is primarily due to the many uncertainties relating to how the evaluation was conducted and how the data might be interpreted. The industry report concludes that based on the different sensory methodologies used (clustering, "In/Out" test and CATA testing), the addition of carob bean at levels up to 0.4% to test cigarettes did not result in a characterizing flavor. Specifically, the report indicates that the test product carob bean "Max" (0.4%) and carob bean "Low" (0.2%) was not describe as having a characterizing flavor and these products are described rather as dried fruits" and "sweetish". Based on cluster analysis, the two test products containing different levels of carob bean were allocated to cluster 11 (carob bean Low, most frequently mentioned attribute "dried tobacco leaves" with a score of 84) and to cluster 12 (carob bean high, most frequently mentioned attribute "dried tobacco leaves" with a score of 90). Both carob bean test products were rated as "Out" by none of the consumers in the "In/Out" test and were therefore not further assessed in the CATA test. Even though the report concludes that conclusions were made based on different sensory methodologies used (*including* CATA testing), it should be noted that only the "In/Out" screening test was used.

The Max-plus concentration was not tested.

There are fundamental methodological flaws with using the "In/Out" test of two products with carob bean concentrations to make the conclusive statement that carob bean additive does not result in a characterizing flavor. Additionally, other vital information is missing from the industry report that could be a determining factor in whether or not carob bean would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which carob bean was incorporated in the tobacco product, as well the quantity of the remaining carob bean. Furthermore, the methods used to select sensory assessors are not considered valid. The reported study used assessors (adult smokers) who were likely to have lower sensitivity to the odor of carob bean in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits (i.e. young, non-smokers). In addition, the screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor, and because the selection criteria identified in advance of testing were often not applied.

Evaluation by the review panel: As a result of the methodological shortcomings, the impact of carob bean on tobacco characterizing flavor is likely to have been underestimated in the reported study. However, even in the case of absence of characterizing flavors, the addition of carob bean can increase the attractiveness of cigarettes and RYO by modifying the perceived flavor, taste or odor of the final product. The industry report does not contain any information about the potential increase of attractiveness on cigarettes and RYO by the addition of carob bean.

5.1.8 Overall conclusion on additive

The industry evaluated carob bean application levels of up to 0.6%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Carob bean is a non-volatile complex mixture consisting of compounds that undergo pyrolysis. Although no new pyrolysis experiments were performed, some of the listed pyrolysis products have CMR properties. The classified carcinogen furfural has been listed as a pyrolysis product but was not included in the comparative testing. Thus, increased CMR properties of the mainstream smoke due to addition of carob bean cannot be ruled out. Inhalation toxicity was not evaluated in the industry report. Given the application level of up to 0.6% and based on the presented data, it cannot be concluded that use of carob bean as an additive in cigarettes and RYO tobacco is without concern regarding toxicity. Influence on inhalation facilitation and addictiveness have not been assessed adequately. Although some pyrolysis products of carob bean are known precursors for MAO inhibitors, no increase was found for analyzed aldehydes at tested levels. A possible decrease of smoke pH due to addition of carob bean that would make the smoke more palatable and possibly easier to inhale has also not been ruled out. Influence on inhalation facilitation thus remains unclear. The impact of carob bean on tobacco flavor is likely to have been underestimated in the reported study and, as carob bean is used to enhance tobacco taste, there is still a concern that it increases the attractiveness of tobacco products, by attenuating bitterness and harshness of tobacco.

The main concerns of the review panel with regards to using carob bean as an additive in cigarettes and roll your own tobacco are the carcinogenicity of some of its pyrolysis products (e.g. furfural), the possible effect on inhalation facilitation, and its flavoring properties that enhance attractiveness.

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5.2 Report of Cocoa

5.2.1 Abstract

Cocoa is a natural substance which is used as an ingredient in the food industry and is used in commercial cigarettes as a tobacco additive in amounts up to 1% of the tobacco weight. Application levels tested in the industry report were 0.5% (Low), 1% (Max), and 1.5% (Max-plus). Cocoa is a solid mixture with some volatile components and variable transfer rates. The pyrolysis products furfuryl alcohol (6.6 %, Carc. 2), furfural (2.1 %, Carc. 2), and phenol (1.6 %, Muta. 2) have CMR properties. The industry concluded in their report that application of cocoa did not result in significant effects regarding smoke chemistry, toxicity, addictiveness, inhalation facilitation or characterizing flavor. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. Regarding toxicity, the provided data are limited and do not eliminate previously identified concerns. Also, more data is needed to determine to which extent cocoa alters the sensory properties of cigarette smoke by providing a more appealing taste, decreasing smoke harshness and thus facilitating inhalation. The main concerns of the review panel with regards to using cocoa as an additive in cigarettes and roll your own tobacco are the carcinogenicity of constituents (e.g. cadmium) and pyrolysis products (e.g. furfural), the possible influence on inhalation facilitation, and its flavoring properties that enhance attractiveness.

5.2.2 Background

Cocoa is a natural compound and a complex mixture of known and unknown chemical components extracted from cocoa beans. Together, these components make up the overall typical flavor and taste of the cocoa extract. Moreover, they can each separately, or interactively together, have addictive, harmful or toxic characteristics. Cocoa has many appearances such as extracts and powders, which are frequently added to cigarettes and other tobacco products. The maximum amount of added cocoa in commercial cigarettes, as declared by manufacturers in the European Common Entry Gate system, is approximately 1% of the total tobacco weight. Previous assessments of scientific data on cocoa as an additive in tobacco products have identified several concerns (1, 2).

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 also not been thoroughly studied and thus, an adequate risk assessment for cocoa or its pyrolysis products is currently not available.

Regarding addictiveness: Several pharmacological effects of cocoa-derived ingredients were reported, including the bronchodilatory effect of theobromine and caffeine, which facilitates deeper inhalation and improved bioavailability of nicotine. Caffeine has also been indicated to have a potential addictive effect on its own, or combined with nicotine (3-7). Furthermore, cocoa is considered a source of constituents, metabolites and pyrolysis products that inhibit monoamine oxidase (MAO) leading to enhanced addictiveness.

Regarding characterizing flavor: Cocoa can alter the sensory properties of cigarette smoke, by providing a more appealing taste and decreasing its harshness (8). However, more data are needed on the amount of cocoa that imparts a noticeable flavor.

5.2.3 Literature review

The industry report provides two literature overviews for cocoa, one regarding cocoa in general and a second one regarding cocoa when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation

toxicity, respiratory sensitization, and toxicity or addictiveness of pyrolysis products (see Section 4.4). The first literature overview contains 20 original researches and 9 review articles, the majority of which (n=18) are from the tobacco industry. Moreover, some important references are missing (i.e. ECHA RAC, 2018 (9)). Relevant issues, such as physiological functions/properties or effects by inhalation are not sufficiently covered.

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.2.4 Chemistry and Pyrolysis products

The report provides a description of cocoa as an additive (CAS 95009-22-6), and relevant specifications about the investigated lot including manufacturer, lot number, and purity (food grade). Further, an analysis of content is provided, confirming that the investigated lot represents the typical characteristics of the additive cocoa powder that is used in cigarettes. It should be noted that it is reported in the industry report that 2.3% of the total content of the cocoa powder consisted of vitamin E, which is striking as the TPD (art. 7.6) states that tobacco products containing added vitamins should be prohibited.

Application levels: The cocoa powder levels applied in test cigarettes varied between 0.5% (Low); 1% (Max); and 1.5% (Max-plus). Actually achieved concentrations after application of cocoa were determined as 0.6%, 1.01%, and 1.44% for the Low, Max, and Max-plus application levels, respectively.

Transfer of cocoa into main stream smoke: Cocoa powder is a complex mixture of compounds that are mainly non-volatile. The industry provided data on the transfer rate into the mainstream smoke only for one compound, namely theobromine. In a previous study from 2017 that was included in the report, the transfer rate of theobromine was found to range between 4 and 5% using ISO method. However, rates of about 13% have been described in earlier reports (10). These differences could be due to blocked filter ventilation. Taken together, it can be concluded that theobromine transfer is likely to be less than 15%. Although not addressed in the industry report, these data provide a useful basis to model a maximum exposure to theobromine by regular smoking. More details on this are presented below under "Additional independent data". No transfer studies were performed for other components of interest, such as caffeine or other products released from cocoa combustion or pyrolysis. However, the content of caffeine in cocoa is relatively low (0.2%).

Pyrolysis experiments: The industry report refers to published studies on pyrolysis of cocoa, most importantly Baker and Bishop, 2005 (11). Pyrolysis products of cocoa powder were acetic acid (27.2%), acetol (6.6%), furfuryl alcohol (6.6%), caffeine (4.0%), pyrrole (2.8%), furfural + cyclopentanone (2.1%), phenol (1.6%), cresol + pyridenediol (1.4%), 2-butanone (0.9%), toluene (0.7%), and styrene (0.2%). Only some of the published pyrolysis products (phenol, toluene, styrene) were tested in the comparative analysis of cigarette smoke, using the ISO Method. Although caffeine is also listed as a pyrolysis product of cocoa, the content in mainstream smoke was not determined. Neither were other pyrolysis products, such as acetic acid, acetol and furfuryl alcohol, even if they are of toxicological concern.

Chemical analysis of mainstream smoke: For comparative testing of main stream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.5 - 1.5 %).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, are described in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of

the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results . Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.

Detailed analysis of the data provided by the industry reveals apparent trends for some smoke constituents that correlate with increasing or decreasing cocoa content. For example, the average level of cadmium is 33% higher in "Max-plus" cigarettes, compared to the reference (no cocoa supplement) or "Low" cigarette. This difference is just below the variability of the 3R4F monitor cigarette (3R4F $Var_{99\%}$ = 34.5%). Other compounds, like 1-aminonaphthalene and quinolone, show substantially decreased levels in correlation with higher cocoa content. This observation raises technical questions that should have been addressed. Notably, sporadic, but significant variations of smoke constituents had also occurred in earlier studies, addressing the effects of cocoa on smoke chemistry (12). Further, moderately reduced toxicant levels (especially TSNAs) that correlated with the content of cocoa have also been demonstrated in independent studies (13). A possible explanation included alterations in the burning process that might have affected transfer rates or replacement of tobacco by other materials.

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). No increase of carbonyls was found at tested levels.

Evaluation by the review panel: The submitted report on cocoa examines the transfer, pyrolysis, as well as the levels of harmful and potentially harmful constituents in cigarette smoke. However, not all aspects of smoke chemistry were covered comprehensively. According to the industry report, cocoa extract is applied up to 1.5% (Max-plus) in tobacco products. It is a complex mixture of compounds, some of them undergo pyrolysis. The industry concluded that no statistically significant and consistent additive-level related increases or decreases were detected for any smoke constituent. An additive-related increase in cadmium was apparent that has not been acknowledged by the industry due to their statistical measures. Moreover, some pyrolysis products of toxicological concern are not evaluated in the industry report. Overall, the use of cocoa as an additive seems to lead to the formation of toxic substances in the tobacco smoke, but the provided data are not sufficient to draw further conclusions.

5.2.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – PARTLY ASSESSSED

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

As discussed in Chapter 4, evaluation of the oral toxicity of a compound has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relative extensive evaluation of the toxicity of cocoa when ingested, based on a literature review focusing on caffeine and theobromine. The report provided by the industry concludes that ingestion of cocoa has no carcinogenic, mutagenic/genotoxic or reprotoxic properties, but does not address the relevance of this conclusion for the evaluation of the toxicity of cocoa when used as a tobacco additive. However, data on chronic and reproductive toxicity in non-rodent species were not presented. While several independent studies have shown toxic and reprotoxic effects of ingestion of caffeine and theobromine at concentrations that are much higher than the doses expected from smoking (14-16).

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

The inhalation toxicity of cocoa is not evaluated in the industry report. Also, information regarding metabolite formation and their possible toxicity is lacking. Since cocoa is composed of a mixture of non-volatile compounds, their transfer in its unburnt form to mainstream smoke is unlikely. However, some constituents of cocoa may be present in the smoke. Thus, an initial step in the assessment of the inhalation toxicity of cocoa itself would be to identify the cocoa-derived compounds transferred in smoke in the industry report. The industry report provides transfer rates for theobromine (4-5%), but not for any other components. Moreover, no evaluation of the inhalation toxicity of theobromine is included.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The pyrolysis products identified in the report were not evaluated in terms of oral or inhalation toxicity. Moreover, the identification of pyrolysis products was based on the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of cocoa were not determined. Based on assessment by the review panel it was concluded that furfural, which was identified as a pyrolysis product in the report, is classified as a CMR carcinogen cat. 2 under the EC Regulation No 1272/2008 (see Annex III – Pyrolysis product table).

D. Evaluation of **mainstream smoke** (comparative testing) – PARTLY ASSESSED

For comparative testing, the report includes both a literature review and new studies. Based on *in vitro* and *in vivo* experiments, that did not identify statistically significant effects, the industry report concludes that an inclusion of 0.5 - 1.5% of cocoa in tobacco does not increase the CMR properties of the mainstream smoke.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion 0.5 - 1.5% of cocoa in tobacco does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. The conclusion of the review panel is that the current report is not sufficient to reach a conclusion regarding the toxicity of cocoa when used as a tobacco additive. There were several methodological limitations in the comparative testing approach, and no data was presented on the inhalation toxicity of cocoa and its pyrolysis products. The pyrolysis product furfural is a classified carcinogen. Increase of furfural levels in mainstream smoke have not been assessed. Thus, a contribution to CMR properties cannot be ruled out.

5.2.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: Cocoa is considered to be a source of constituents, metabolites, and pyrolysis products that inhibit monoamine oxidase (MAO). As monoamine oxidase inhibitors (MAOI) have been shown to potently increase addictiveness of nicotine (17, 18) any compound giving rise to MAOI would therefore increase the addictiveness of nicotine.

Inhalation facilitation: two of cocoa's main constituents (theobromine, caffeine) are believed to possess bronchodilatory effects, which facilitate deeper inhalation and improved bioavailability of nicotine (1, 2). Moreover, decrease of smoke pH leads smokers to "increase their smoking frequency and inhale the smoke more deeply to enable a higher absorption of nicotine in the airways", as well as decreases the harshness and irritation of the smoke encouraging them "to develop a smoking habit" (19). The industry report indicates that pyrolysis of cocoa leads to the formation of acids. Therefore, addition of cocoa can potentially decrease smoke pH and could then reduce harshness of smoke and facilitate inhalation.

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of cocoa, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added cocoa to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of cocoa on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from cocoa. Specifically, none of the reported studies did asses the capacity of metabolites and pyrolysis products of cocoa on monoamine oxidase inhibition. There were also no studies reported assessing the effects of cocoa on nicotine bio-availability and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores.*

There were no tests reported to assess bronchodilating effects of the added amount of cocoa (on its own and in the product), or its constituents. In addition, there is no reference to the potential addictive effect of caffeine or the combination caffeine + nicotine which could reveal an addictive-like profile (3-7).

Evaluation by the review panel: The provided data show no effect of adding cocoa on inhalation facilitation and nicotine uptake, but these data are limited and do not address the previously identified concern regarding MAO inhibition and bronchodilation. In the independent re-evaluation of the chemical analysis of mainstream smoke data reported in the industry report (see Section 4 of this Chapter and Annex II), no increase of carbonyls was found at the tested levels. Other compounds that might be relevant for inhalation facilitation, nicotine delivery or bronchodilating effects were not assessed in the industry report. The fact that independent data show that the main constituents of cocoa may enhance the reinforcing effects of nicotine through MAO inhibition and reduce the harshness of smoke by decreasing its pH raises some concern regarding addictiveness and inhalation facilitation. Altogether, there is insufficient evidence to rule out an influence on addictiveness or inhalation facilitation upon application of cocoa as an additive in cigarettes and RYO tobacco.

5.2.7 Characterizing flavor

Although cocoa is a known flavoring agent, it is not possible to conclude whether the additive cocoa can be responsible for introducing a characterizing flavor or odor to tobacco products, on the basis of the evidence presented in the industry report. This is primarily due to the many uncertainties

relating to how the evaluation was conducted and how the data might be interpreted. The industry report concludes that based on the different sensory methodologies used (clustering, "In/Out" test and CATA testing), the addition of cocoa at levels up to 1.0% to test cigarettes did not result in a characterizing flavor. Specifically, the report indicates that the test product cocoa "Max" (1.0%) and cocoa "Low" (0.5%) was not describe as having a characterizing flavor. Based on cluster analysis, the two test products containing different levels of cocoa were allocated to cluster 11 (cocoa "Max", most frequently mentioned attribute "dried tobacco leaves" with a score of 84) and to cluster 8 (cocoa "Low", most frequently mentioned attribute "dried tobacco leaves" with a score of 94). Both cocoa test products were rated as "Out" by none of the consumers in the "In/Out" test and were therefore not further assessed in the CATA test. Even though the report concludes that conclusions were made based on different sensory methodologies used (*including* CATA testing), it should be noted that only the "In/Out" screening test was used. The Max-plus concentration is not tested.

There are fundamental methodological flaws with using the "In/Out" test of two products with cocoa concentrations to make the conclusive statement that cocoa additive does not result in a characterizing flavor. Additionally, other vital information is missing from the industry report that could be a determining factor in whether or not cocoa would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which cocoa was incorporated in the tobacco product, as well the quantity of the remaining cocoa. Furthermore, the methods used to select sensory assessors are not considered valid. The reported study used assessors (adult smokers) who were likely to have lower sensitivity to the odor of cocoa in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits (i.e. young, non-smokers). In addition, the screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor, and because the selection criteria identified in advance of testing were often not applied.

Evaluation by the review panel: Even in the case of absence of characterizing flavors, the addition of cocoa can increase the attractiveness of cigarettes and RYO by modifying the perceived flavor, taste or odor of the final product. Industry report does not contain any information about the potential increase of attractiveness on cigarettes and RYO by the addition of cocoa. The impact of cocoa on tobacco flavor is likely to have been underestimated in the reported study.

5.2.8 Additional information based on independent data sources – as retrieved by WP 9 participants

Conclusions about the dependence potential of caffeine and theobromine found in independent literature are not presented in the report. Independent studies on skin/respiratory sensitization and addictiveness do exist, but were not included and interpreted. In fact, we found studies in other databases like SCOPUS/PUBMED, ECHA and NTP that reported on caffeine and theobromine that were highly relevant and contradict the statements above. For instance, Smith *et al.* stated that: "Of all constituents proposed to play a role in our liking for chocolate, caffeine is the most convincing, though a role for theobromine cannot be ruled out."(20). Furthermore, he stated "identical improvements on the mood construct "energetic arousal" and cognitive function were found for cocoa powder and the caffeine + theobromine combination versus placebo"(21). Moreover, another study of Napierala *et al.* stated that "nicotine and caffeine affect the release of dopamine. It is believed that the interaction of these substances may be a synergistic effect […]."(22).

5.2.9 Overall conclusion on additive

The industry evaluated cocoa application levels of up to 1.5%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of

the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Cocoa is a non-volatile complex mixture consisting of compounds that undergo pyrolysis. Although no new pyrolysis experiments were performed, some of the listed pyrolysis products had CMR properties. The classified carcinogen furfural has been listed as a pyrolysis product, but was not included in the comparative chemical analysis. Thus, increased CMR properties of the mainstream smoke due to addition of cocoa in cigarettes cannot be ruled out. Levels of cadmium were increased in a dose-dependent manner that almost exceeded the 99% variability of monitor cigarettes (34.5%). Inhalation toxicity was not evaluated in the industry report. Thus, given the application level of up to 1.5% and based on the presented data, it cannot be concluded that use of cocoa as an additive in cigarettes and RYO tobacco is without concern regarding toxicity.

The possible influence of cocoa on inhalation facilitation and addictiveness have not been assessed adequately in the industry reports. Bronchodilatory effects of transferred theobromine have neither been assessed nor discussed. Although some pyrolysis products of cocoa are known precursors for MAO inhibitors, no increase was found for analyzed aldehydes at tested levels. The transfer rate of caffeine to mainstream smoke and its possible contribution to nicotine reinforcing effects have not been assessed. Possible decrease of smoke pH due to addition of cocoa that would make inhalation more palatable has not been ruled out. Influence on inhalation facilitation remains unclear. The impact of cocoa on tobacco flavor has likely been underestimated in the reported study and, as cocoa is used to enhance tobacco taste, there is still a concern that it increases the attractiveness of tobacco products by attenuating bitterness and harshness of tobacco.

The main concerns of the review panel with regards to using cocoa as an additive in cigarettes and roll your own tobacco are the carcinogenicity of some of its constituents (e.g. cadmium) and pyrolysis products (e.g. furfural), its possible influence on inhalation facilitation, and its flavoring properties that enhance attractiveness.

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5.3 Report of Fenugreek

5.3.1 Abstract

Fenugreek modifies the flavor in food and is used in commercial cigarettes as a tobacco additive in amounts up to 0.02 % of the tobacco weight. Application levels in the industry report were 0.01% (Low), 0.02% (Max), and 0.03% (Max-plus). Fenugreek is a complex mixture of mainly non-volatile compounds. A transfer of these compounds into mainstream smoke is unknown but unlikely and has not been studied. The reported pyrolysis products properties benzene (0.2% Muta. 1b, Carc. 1a), toluene* (0.2%, Repr. 2), 2-butenal (0.1% Muta. 2), and furfural (0.03-0.3%, Carc. 2) have CMR properties. The industry concluded in their report that application of fenugreek extract did not result in significant effects regarding smoke chemistry, toxicity, addictiveness, inhalation facilitation or characterizing flavor. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. The main concerns of the review panel with regard to using fenugreek extract as an additive in cigarettes and roll your own tobacco are the CMR properties of some pyrolysis products and its flavoring properties that enhance attractiveness.

5.3.2 Background

Fenugreek (CAS number: 84625-40-1) is a natural seed-extract comprising a complex mixture of a number of chemical compounds (of which about 70 have been identified) variably contributing to the taste and flavor of tobacco smoke. Overall, fenugreek compounds can individually or synergistically contribute to the harmfulness, addictiveness, attractiveness and toxicity of tobacco smoke. Several health concerns based on previous research related to fenugreek extract as tobacco additive have been identified:

Regarding toxicity: Adverse health effects such as CNS stimulant and depressant activities, allergic reactions, and gastrointestinal discomfort have been reported after fenugreek extract (used as spice in food or as medicine) ingestion, inhalation or external application (1-4). There is increasing evidence that fenugreek extract may have neurodevelopmental, neurobehavioral and neuropathological side effects (2). Fenugreek extract is a skin irritant (5). When heated to decomposition, it produces acrid smoke and irritating fumes. Combustion pyrolysis (up to 900°) of fenugreek can produce a number of toxic, carcinogenic, mutagenic, and aroma compounds (6-8).

Regarding addictiveness: Fenugreek extract and its pyrolysis products include smoothing agents, flavors (such as caramel), and compounds that can enhance nicotine addictive properties such as aldehydes, which are potent monoamine oxidases (MAO) inhibitors (4, 9).

Regarding characterizing flavor: Fenugreek extract modifies flavor in several food products in much lower contents than those used in tobacco products. Fenugreek extract polysaccharides improve the taste of cigarette smoke by giving a characterizing flavor and aroma to tobacco and attenuating the alkaloid bitterness and harshness (4, 10). Combustion products include pyrazines that are formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. They give the tobacco product an attractive smell and taste, thus increasing product appeal, promoting smoke inhalation (4, 8, 11, 12).

5.3.3 Literature review

The industry report provides two literature overviews for fenugreek extract, one regarding the additive in general and a second one regarding fenugreek extract when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an

* not distinguishable from another (non-CMR) compound in the pyrolysis experiment

underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4).

One overview consists of studies on fenugreek extract toxicological properties, all funded by the tobacco industry (n=16, of which 15 original articles and 1 review article). Independent studies available in the scientific literature are not included. We highlight for example that research on skin and eye irritation and possible respiratory sensitization from pyrolysis products from extract or oil was not included. Moreover, no information on addictiveness/attractiveness was reported in the industry report, although according to other sources retrieved by the review panel, the characterizing flavor, to which the volatile constituent 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolone) markedly contributes (13), can possibly influence attractiveness of the product. Another study on fenugreek extract as tobacco additive reported that cut tobacco moisture ability was stronger with fenugreek extract polysaccharides than with samples of propylene glycol in an environment of low relative humidity. Fenugreek extract polysaccharides had moisture-proof effect at a moderately high humidity and adding 0. 005% - 0.1% of cut tobacco weight showed the most prominent flavoring effect (10).

Some findings and shortcomings of the industry's literature search are discussed below in the relevant sections or at the end of the report. Taken together, the literature overview provided by industry is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.3.4 Chemistry and Pyrolysis products

According to the industry report, fenugreek extract is used as flavoring in commercial tobacco products up to a maximum content of 0.02%. Information on the tested products, including lot specification and analytical data on individual constituents are provided in the industry report. It should be kept in mind that under REACH, fenugreek extract is identified as an UVCB (Unknown or Variable composition, Complex reaction products or Biological materials) and that this additive can vary in its composition. Fenugreek extract contains comparatively high levels of mono- and polyunsaturated fatty acids (i.e. linoleic acid, palmitoleic acid).

Application levels: The fenugreek extract levels applied in test cigarettes varied between 0.01% and 0.03% (Low 0.01%; Max, 0.02%; Max-plus 0.03%). Actually achieved concentrations after application of fenugreek extract have not been tested ("No analysis method available"). The target value of fenugreek in the "mix " 1 cigarette was 0.02%.

Transfer of fenugreek into mainstream smoke: Based on the non-volatile properties of the major constituents, the industry concludes that transfer of intact molecules into mainstream smoke is very unlikely. In addition, fenugreek extract is a complex additive, consisting of numerous compounds that have been shown to undergo pyrolysis. However, no further experimental work has been performed to identify the transfer of fenugreek extract substances to MSS.

Pyrolysis experiments: The industry report refers to published studies on pyrolysis of fenugreek extract, fenugreek oil and fenugreek tincture, most importantly from Baker and Bishop (14). Major pyrolysis products of fenugreek extract include ethyl linoleate (37.4 %), ethyl palmitate (14.8 %), ethyl stearate (10.6 %), palmitic acid (6 %) and hydroxydimethylfuranone (3.4 %), while the pyrolysis of fenugreek oil produces diethyl tartrate (74.3 %), acetic anhydride (4.7 %), acetic acid (4.7 %), ethyl oleate (3.9 %) and ethyl linoleate (2.4 %). The pyrolysis of fenugreek tincture produces heptanoic acid (71.4 %), acetic acid (5.2 %), pyridine (3.4 %) vinylphenol (2.5 %), phenol (2.3 %) and furfural (0.5 %).

Chemical analysis of mainstream smoke: The literature search performed by the tobacco industry reports increased emissions of particulate matter (15) and of several compounds (such as formaldehyde, acetaldehyde and other carbonyls, NH3, PAH, heavy metals, etc.) when fenugreek

extract is used as an additive alone (3) or in combination with other additives, compared to reference cigarettes (15-18). A statistically significant increase in the emission of formaldehyde is reported (19). However, due to the fact, that fenugreek extract was only one of additives used in that last study, it is difficult to attribute the increase of formaldehyde to fenugreek extract exclusively.

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, is included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results . Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.

In the industry experiments, the comparative testing approach showed some differences between the test cigarettes and the additive free reference product. The highest increases of analytes were observed among the tobacco specific nitrosamines (TSNA) in the "Max" cigarette (0.02%), with increase of NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) exceeding the variability of 3R4F reference cigarette. The "Max-plus" addition of fenugreek extract (0.03%) reversed this observation, with no clear explanation. Other compounds show sporadically higher emissions (HCN, phenols, 1,3 butadiene, toluene,…), without clear explanation for their variations. The "mix 1" cigarette, containing fenugreek extract and other 5 additives, showed an increase in the emissions of some aromatic amines, several carbonyls, NNK, Cd, NH3, NOx, and isoprene, but this increase cannot be attributed to fenugreek extract exclusively. The statistical analysis performed by the industry did not result in any overall statistically significant increase or decrease of any of the tested chemical compounds other than water.

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). No increase of carbonyls was found at the tested levels.

Evaluation by the review panel: The submitted report on fenugreek extract examines the transfer, pyrolysis, as well as the levels of harmful and potentially harmful constituents in cigarette smoke. However, no new pyrolysis experiments were performed and there are limitations in the comparative testing approach. Fenugreek extract is however applied in very low levels (< 0.05%). The industry concluded that no statistically significant and consistent additive-level related increases or decreases were recorded for any smoke constituent. Although, there were several limitations in the approach and methodology, an influence on smoke chemistry from the application of fenugreek extract as an additive in cigarettes and RYO tobacco is unlikely, given its low application level, its decomposition during the pyrolysis phase and the level of changes in emissions of the chemical compounds analyzed comparing to reference cigarettes, but cannot be ruled out.

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

5.3.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – ASSESSED

The industry report includes a relatively extensive evaluation of the toxicity of fenugreek extract when ingested. Fenugreek extract is widely used in many consumer goods, such as foods, and in medicinal products so there is a long history of oral exposure to this additive (20). However, as discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. Fenugreek extract levels that are relevant to food are not expected to have detrimental effects with respect to reproduction and development in humans.

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

The inhalation toxicity of fenugreek extract is not evaluated in the industry report, thus the possibility that addition of fenugreek may increase the irritating effects of cigarette smoke to respiratory organs cannot be excluded. There are reported cases of allergic reactions after inhalation and external application of fenugreek extract seed powder (2), but fenugreek extract is not considered a skin or respiratory sensitizer according to the industry report, although this claim is not substantiated by relevant toxicological data. It should be noted that ECHA has classified fenugreek extract as a skin irritant (5). Also, information regarding metabolites formation and their possible toxicity is lacking. Since fenugreek extract is not a volatile compound, transfer in its unburnt form to mainstream smoke is not likely. Whether fenugreek extract in heated but unburned form, or components from fenugreek extract decomposed by burning, will have effects via inhalation of cigarette smoke is not assessed in the report.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The pyrolysis products identified in the report were not evaluated in terms of oral or inhalation toxicity. Moreover, the identification of pyrolysis products was based on the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of fenugreek extract were not determined. Based on assessment by the review panel it was concluded that four of the reported pyrolysis products had CMR properties (Benzene Muta. 1b, Carc. 1a; toluene* Repr. 2; 2-butenal, Muta. 2; Furfural Carc. 2). Further, some pyrolysis products, like 2-butanone, pentanol ,acetic anhydride, acetic acid, and heptanoic acid, are classified or suspected to cause skin or eye irritation or specific target organ toxicity (see Annex III – "Pyrolysis product table").

E. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

For comparative testing, the report includes both a literature review and new studies. Based on comparative *in vitro* and *in vivo* experiments that did not identify significant effects on toxicity, the industry report concludes that addition of up to 0.03% of fenugreek extract in tobacco does not increase the CMR properties of the main stream smoke.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

* not distinguishable from another (non-CMR) compound in the pyrolysis experiment.

Evaluation by the review panel: Oral ingestion is not relevant for the evaluation of the toxicity of inhaled fenugreek extract, and this tobacco additive is not an authorized food additive in the EU. In food it is associated with a number of biological effects, some of these being possibly considered as beneficial (anabolic and androgenic activity i.e. with endocrine effects) but this depends on the dose ingested. In addition, it is not clear how the extracts are prepared that are used in tobacco, which further limits the comparison with intake of fenugreek extract as a food ingredient. Therefore, the statement by industry that "fenugreek extract has a long and established history of safe use as food ingredient" is not relevant for the evaluation of fenugreek extract as a tobacco additive. In addition, the report does not evaluate the toxic, genotoxic and carcinogenic potential of the products formed by pyrolysis of fenugreek extract as such. Some of the reported pyrolysis products (Benzene , Muta. 1b, Carc. 1a; toluene*, Repr. 2; 2-butenal, Muta. 2; Furfural Carc.2) have CMR properties. Therefore, the review panel concludes that a contribution to increased CMR properties upon application of fenugreek as an additive in cigarettes and RYO tobacco cannot be ruled out, in spite of its low application and transfer rate.

5.3.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: Concerns were raised (SCENIHR opinion 1 (4)) that the content and pyrolysis products from fenugreek extract include substances that potentiate addictive effects of nicotine through the process of MAO inhibition (e.g. aldehydes).

Inhalation facilitation: The content and pyrolysis products from fenugreek extract include smoothing agents (e.g. organic acids), which may reduce the harshness of smoke and facilitate inhalation (4).

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of fenugreek extract, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added fenugreek extract to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of fenugreek extract on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from fenugreek extract. Specifically, none of the reported studies did asses the capacity of metabolites and pyrolysis products of fenugreek extract on monoamine oxidase inhibition. There were also no studies reported assessing the effects of fenugreek extract on nicotine bio-availability and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores.*

Evaluation by the review panel: The provided data show no effect of adding fenugreek extract at tested levels on inhalation facilitation and nicotine uptake, but these data are limited and do not address the previously identified concern regarding MAO inhibition. In the independent re-evaluation of the chemical analysis of mainstream smoke data reported in the industry report (see Section 4 of this Chapter and Annex II), no increase in carbonyl emissions at the tested levels was detected. Other compounds relevant for inhalation facilitation or nicotine delivery were not assessed in the industry report. Altogether, there is insufficient evidence to rule out an influence on addictiveness of inhalation facilitation upon application of fenugreek as an additive in cigarettes and RYO tobacco.

* not distinguishable from another (non-CMR) compound in the pyrolysis experiment.

5.3.7 Characterizing flavor

Although fenugreek extract is a known flavoring agent, it is not possible to conclude whether the additive fenugreek extract can be responsible for introducing a characterizing flavor or odor to tobacco products, on the basis of the evidence presented in the industry report. This is primarily due to the many uncertainties relating to how the evaluation was conducted and how the data might be interpreted. The industry report concludes that based on the different sensory methodologies used (clustering, "In/Out" test and CATA testing), the addition of fenugreek extract at levels up to 0.02% to test cigarettes did not result in a characterizing flavor. Specifically, the report indicates that the test product fenugreek extract "Max" (0.02%) was described by the sensory panelists (all adult smokers) as having a "curry" note, while fenugreek extract "Low" (0.01%) was described as having the attributes "sweetish" and "dried fruits". Based on cluster analysis, the two test products containing different levels of fenugreek extract were allocated to cluster 10 (most frequently mentioned attribute "dried tobacco leaves" with a score of 75, cluster scoring somewhat higher for "curry" with a score of 30 and for "licorice" with a score of 25 than other clusters). Both fenugreek extract test products were rated as "Out" by none of the consumers in the "In/Out" test and were therefore not further assessed in the CATA test. Even though the report concludes that conclusions were made based on different sensory methodologies used (*including* CATA testing), it should be noted that only the "In/Out" screening test was used.

There are fundamental methodological flaws with using the "In/Out" test of two products with fenugreek extract concentrations to make the conclusive statement that fenugreek extract additive does not result in a characterizing flavor. Additionally, other vital information is missing from the industry report that could be a determining factor in whether or not fenugreek extract would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which fenugreek extract was incorporated in the tobacco product, as well the quantity of the remaining fenugreek. Furthermore, the methods used to select sensory assessors are not considered valid. The reported study used assessors (adult smokers) who were likely to have lower sensitivity to the odor of fenugreek extract in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits (i.e. young, non-smokers). In addition, the screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor, and because the selection criteria identified in advance of testing were often not applied.

Evaluation by the review panel: It should be noticed that fenugreek extract used to modify flavor in food is usually from 2 to 200-300 ppm (0.002-0.003%), except condiments which can be up to 4,000 ppm (0.4%). Fenugreek extract is added in tobacco in contents 0.01 to 0.03% in the comparative tests, but only up to 0.02% in the sensory tests. As a result of the methodological shortcomings, the impact of fenugreek extract on tobacco flavor is likely to have been underestimated in the reported study. However, even in the absence of a characterizing flavor, the addition of fig can increase the attractiveness of cigarettes and RYO by modifying the perceived flavor, taste or odor of the final product. Moreover, as fenugreek extract is a natural product used in food since the antiquity and was also used for medicinal purposes in the past, it can give the impression of a product having health benefits or having decreased health impacts.

5.3.8 Overall conclusion on additive

The industry evaluated fenugreek extract application levels of up to 0.03%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions

based on the information provided in the industry reports and independent literature.

Fenugreek extract is applied in low levels in tobacco (≤0.03%) and is a non-volatile complex mixture consisting of compounds that undergo pyrolysis. No new pyrolysis experiments were performed, however some of the listed pyrolysis products have CMR (Benzene , Muta. 1b, Carc. 1a; toluene*, Repr. 2; 2-butenal, Muta. 2; Furfural Carc.2) or irritative properties. Although some pyrolysis products of fenugreek extract are known precursors for MAO inhibitors, no increase was found for analyzed carbonyls at the tested levels. Inhalation toxicity was not evaluated in the industry report. The review panel concludes that it cannot be ruled out that use of fenugreek as an additive contributes to increased CMR properties of mainstream smoke. In addition, allergic and irritative effects of fenugreek extract cannot be ruled out. Another issue is whether fenugreek extract may give a characterizing flavor and aroma to tobacco. The impact of fenugreek extract on tobacco flavor is likely to have been underestimated in the reported study and, as fenugreek extract is used to enhance tobacco taste, there is still a major concern that it will increase the attractiveness of tobacco products, by attenuating bitterness and harshness of tobacco.

The main concerns of the review panel with regard to using fenugreek extract as an additive in cigarettes and roll your own tobacco are the CMR properties of some pyrolysis products, its potential to contribute to allergy or irritations and its flavoring properties that enhance attractiveness.

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5.4 Report of Fig

5.4.1 Abstract

Fig concentrate has a rich carbohydrate/sugar content, together with varying amounts of proteins, amino acids and other flavoring compounds. It has a fruity characteristic flavor and it is used as an additive to smooth cigarette smoke and to improve the aroma of tobacco. The amount of fig concentrate applied in the test cigarettes is from 0.025% (Low) to 0.15% (Max) and 0.3% (Max-plus). Fig is a complex mixture of mainly non-volatile compounds. A transfer of these compounds into mainstream smoke is unknown but unlikely and has not been studied in the industry report. The reported pyrolysis product furfural (24.5 %) has CMR properties (Carc. 2). The industry concluded in their report that application of fig did not result in significant effects regarding smoke chemistry, toxicity, addictiveness, inhalation facilitation or characterizing flavor. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. The main concerns of the review panel with regard to using fig as an additive in cigarettes and roll your own tobacco is the carcinogenicity of pyrolysis products (furfural) and its flavoring properties that enhance attractiveness.

5.4.2 Background

Fig juice concentrate (Ficus carica Linn. extract, CAS number: 90028-74-3) is a complex mixture, high in natural monosaccharides glucose and fructose. Fig concentrate has a rich carbohydrate/sugar content, together with varying amounts of proteins, amino acids and other flavoring compounds. The amount of fig juice concentrate used in tobacco products is not provided in the industry report, but SCENIHR reports the use of fig juice concentrate by tobacco manufacturers as flavoring at <0.0001 % and at 0.0022 % w/w (1).

Several health concerns based on previous research related to fig extract have been identified:

Toxicity: Effects by inhalation and chemistry of combustion/pyrolysis of fig concentrate are not yet sufficiently characterized in terms of physiological, toxicological and synergistic effects to potentiate the harmful effects of tobacco smoke. Studies should be performed to identify the compounds produced from burning fig juice concentrate. Combustion products from the high sugar/carbohydrate concentration possibly include aldehydes and toxic carcinogenic compounds (1). Fig extract is a highly flammable liquid and vapor, that causes skin irritation, serious eye irritation, and may cause allergic reactions (2). An adequate risk assessment for fig extract or its pyrolysis products is currently not available.

Addictiveness: Combustion and pyrolysis of sugars form compounds such as aldehydes, and mainly acetaldehyde. They can contribute to the formation of MAO inhibitors and may increase addictiveness to nicotine (3).

Characterizing flavor/Attractiveness: Fig concentrate has a fruity characteristic flavor (1). Studies using ficus carica as flavoring additive in cigarettes, reported that the cigarette smoke was smoother and mellower, the aroma and the smoking quality was improved (4, 5).

5.4.3 Literature review

The industry report provides two literature overviews for fig, one regarding the additive in general and a second one regarding fig when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4).

One overview consists of 12 studies, of which 11 were funded by the tobacco industry (10 original articles and 1 review article).

With respect to fig oral ingestion toxicity, the industry reports the following conclusion: “The constituents of figs are food constituents that form part of the normal diet of humans, and, as such, is very well tolerated”. However, this conclusion raises doubts in light of what the literature review of Barolo *et al.* (6) reported: “Despite the security offered as food, some authors consider that the toxicological evaluation of fig products is still in an early stage”. Fig is a food ingredient not authorized as a food additive in the EU.

With regard to fig concentrate´s contribution to addictiveness, facilitation of inhalation and facilitation of nicotine uptake, the industry concludes: “On searching the scientific literature, no data was found investigating the ability of fig extract to reduce the harshness or smoothen tobacco smoke”. However, in an independent search, the review panel identified studies reporting research on aroma components in essential oil from *ficus carica* and its application in cigarette flavoring (4) and on preparation of characteristic tobacco flavors from *Ficus carica* by fermentation (5). The purpose was to increase product appeal, with a more attractive smell and taste, thus promoting the repetition of smoking inhalation and possibly influencing addictiveness by increasing the level of nicotine intoxication.

Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.4.4 Chemistry and Pyrolysis products

The report provides a brief description of the additive (CAS number 90028-74-3), as well as lot properties and a list of constituents confirming a high content in carbohydrates, sugars and water. Concentrated juice derived from the fruit of the fig tree (*Ficus carica*) as used in the tobacco industry was used. The submitted data includes information on the manufacturer and compliance with applicable EU food flavoring legislation and manufacture of the test cigarettes.

Application levels: The fig levels applied in test cigarettes varied between 0.025 % and 0.3 % (Low 0.025 %; Max, 0.15 %; Max-plus 0.3 %). However, the concentration used in commercial products is not specified, and achieved concentrations after application of fig have not been tested ("Technically not possible to detect achieved amount").

Transfer of fig into mainstream smoke: Fig juice concentrate is a complex additive, consisting of numerous compounds, mainly sugars and carbohydrates that have been shown to undergo pyrolysis. Based on the complexity and non-volatile nature of constituents and preliminary indications of degradation during pyrolysis, the industry concluded that intact materials or molecules are unlikely to transfer into mainstream smoke. Consequently, transfer rates have not been determined.

Pyrolysis products: The industry report takes reference to a published study on pyrolysis of fig juice concentrate (7). The major pyrolysis products include acetic acid (45.1 %), furfural (24.5 %), sorbic acid (10.2 %), butanediol (3.7 %), as well as an unidentified compound (8.6 %). Further, the report summarizes that pyrolysis conditions only approximate combustion of a burning cigarette and data are not predictive for smoke chemistry for the non-volatile additives. Consequently, new pyrolysis experiments were not conducted.

Chemical analysis of mainstream smoke: For comparative testing of main stream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.025 to 0.3 % fig juice).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, is included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results . Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

In the industry experiments, the comparative testing approach showed some differences between the test cigarettes and the additive free reference product. The highest increase of analytes was observed among the tobacco specific nitrosamines (TSNA), with increase of NNN (N'-nitrosonornicotine) at "Max-plus" level and NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) at "Max" level exceeding the variability of the 3R4F reference cigarette. The increase in water exceeded the variability of the 3R4F reference cigarette at "Max" levels. Overall, the statistical analysis did not result in any overall statistically significant increase or decrease of any of the tested chemical compounds other than water.

Taken together, the industry report on fig concludes that there are no statistically significant and consistent additive related effects on the composition of smoke. However even when using a 99% confidence interval some compounds are found to be out of this interval (NNK, NNN and water). However, a high standard deviation was observed for these levels, indicating that the data is not of sufficient quality to draw sound conclusions. Further discussions and assessments of internal or published historical data could have provided further clarification.

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). No increase of carbonyls was found at tested levels.

Evaluation by the review panel: The submitted report on fig examines pyrolysis and the levels of harmful and potentially harmful constituents in cigarette smoke. However, no new pyrolysis experiments were performed and there are limitations in the comparative testing approach. According to the industry report, fig juice is applied up to 0.3% (Max-plus) in the tobacco products tested. It is a complex mixture of compounds that undergo pyrolysis. The industry concluded that no statistically significant and consistent additive-level related increases or decreases were recorded for any smoke constituent. Although there were limitations in the applied approach and methodology, the members of the review panel conclude that, for the analyzed smoke constituents, significant additive-level related effects on smoke chemistry after addition of fig juice at tested levels are unlikely, but cannot be ruled out. However, there may be an influence of other compounds not analyzed here, such as the previously identified pyrolysis products of fig; furfural and acetic acid.

5.4.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – (PARTLY) ASSESSED

As discussed in Chapter 4, evaluation of the oral toxicity of a compound has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relatively extensive evaluation of the toxicity of fig when ingested. The industry stated that "the constituents of figs are food constituents that form part of the normal diet of humans, and, as such, are well tolerated in general." (*Industry report on Fig, p. 15*).

In rats and mice, the acute oral toxicity of fig extracts is low (no toxicity at doses up to 2000 mg/kg bw). However, ethanol extracts of fig tested at 100 and 200 mg/kg in a single acute toxicity study showed increased urine volume and excretion (8). No long term (90 days) studies are presented. In a 21 day repeated dose rat study (doses ≤ 1000 mg/kg), originally designed for testing antihypertensive and cardio-inhibitory effects, no adverse effects of the extract were noted (9).

The consulted scientific literature demonstrates that fig juice is neither carcinogenic, mutagenic/genotoxic nor toxic to reproduction. It should be noted that single entries to the classification and labelling inventory have listed fig extract as skin sensitizer (category code: 1; hazard code: H317),

irritating to the skin (category code: 2; hazard code: H315) and irritating to the eye (category code: 2; hazard code: H319). Allergic potential is not assessed.

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

The possibility that addition of fig extracts may increase the irritating effects of cigarette smoke to respiratory organs cannot be excluded. The industry report stated that the transfer of the intact additive was not likely, based on the chemical composition, the non-volatile nature of the juices, and the detection of some degradation products. Specific studies on inhalation were not presented, and only a sentence claiming that: "fig juice is not likely to enhance the toxicity of cigarette" was provided.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The pyrolysis products identified in the report were not evaluated in terms of oral nor inhalation toxicity. Moreover, the identification of pyrolysis products was based on literature applying the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of fig were not determined. The review panel performed an independent toxicological assessment of the pyrolysis products provided by the industry (see Annex III – "Pyrolysis product table"). One example of toxicologically relevant information that is not provided in the report is that furfural, which was identified as a pyrolysis product in the report, is classified as a CMR carcinogen cat. 2 under the EC Regulation No 1272/2008 (10). Therefore, it is likely to contribute to the toxicity of the tobacco product. Further, the pyrolysis product acetic acid is classified as a skin corrosive.

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

For comparative testing, the report includes both a literature review of *in vitro* and *in vivo* studies and new *in vitro* studies. In the literature review, mainstream smoke generated from test cigarettes with fig juice applied at up to 11,700 ppm had no discernible effect on the overall toxicity compared to mainstream cigarette smoke from test cigarettes without additives in rats after a 90-day inhalation exposure (11-19). Moreover, the tumorigenicity of condensate prepared from cigarettes containing a number of additives in combination, including fig juice at a concentration up to 5 ppm, was not indicative of any clear effect on the tumorigenicity of cigarette smoke condensate, when compared to control cigarettes without additives.

Several studies, conducted according to the OECD Principles of Good Laboratory Practice and performed following relevant Test Guidelines, have evaluated the mutagenicity, genotoxicity and cytotoxicity of cigarette smoke, in test cigarettes containing fig juice added as part of a mixture of ingredients, added to a standard, reference tobacco blend. Authors in all reported studies concluded that there were no statistically significant differences in the mutagenicity, genotoxicity, and cytotoxicity of cigarette smoke generated from test cigarettes containing fig juice (CAS number 68916-52-9) up to 11,700 ppm, when compared to control reference cigarettes to which no ingredients were added. Also, in the newly performed studies, there were no significant effects for the test cigarettes containing fig juice concentrate. Thus, the industry concluded that addition of 0,3% fig does not affect the CMR properties of cigarette smoke.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 0.025-0.3 % fig juice does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, the review panel concludes that the data presented in the industry report are not sufficient

to reach a conclusion regarding the toxicity of fig when used as a tobacco additive. That is, there were several methodological limitations in the comparative testing approach. Furthermore, only CMR properties were considered and no data was presented on the inhalation toxicity of fig and its pyrolysis products. For instance, the pyrolysis product furfural is a classified carcinogen, and a possible increase of furfural levels or other pyrolysis products like acetic acid in mainstream smoke has not been assessed. Thus, a contribution to CMR properties cannot be ruled out.

5.4.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: Concerns were raised (SCENIHR opinion 1) that the content and pyrolysis products from fig include substances that potentiate addictive effects of nicotine through the process of MAO inhibition (e.g. aldehydes) (1).

Inhalation facilitation: The content and pyrolysis products from fig include smoothing agents (e.g. organic acids), which may reduce the harshness of smoke and facilitate inhalation (SCENIHR opinion 1) (1).

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of fig, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added fig to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of fig on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from fig. Specifically, none of the reported studies did asses the capacity of metabolites and pyrolysis products of fig on monoamine oxidase inhibition. There were also no studies reported assessing the effects of fig on nicotine bio-availability and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores.*

Evaluation by the review panel: The provided data show no effect of adding fig at tested levels on inhalation facilitation and nicotine uptake, but these data are limited and do not address the previously identified concern regarding MAO inhibition. In the independent re-evaluation of the of mainstream smoke data reported in the industry report (see Section 4 and Chapter 3), no increase in carbonyl emissions at the tested levels was detected. Other compounds that might be relevant for inhalation facilitation or nicotine delivery were not assessed in the industry report. Altogether, there is insufficient evidence to rule out an influence on addictiveness or inhalation facilitation upon application of fig as an additive in cigarettes and RYO tobacco.

5.4.7 Characterizing flavor

Although fig is a known flavoring agent, it is not possible to conclude whether the additive can be responsible for introducing a characterizing flavor or odor to tobacco products, on the basis of the evidence presented in the industry report. This is primarily due to the many uncertainties relating to how the evaluation was conducted and how the data might be interpreted.

The industry report concludes that based on the different sensory methodologies used (clustering, “In/Out” test and CATA testing), the addition of fig juice concentrate at levels up to 0.15% to test cigarettes did not result in a characterizing flavor. Both test products containing fig extract were described by the sensory panelists (all adult smokers), as having a “sweetish” note. Specifically, the report indicates that fig “Low” (0.025%) was allocated to cluster 7 (most frequently mentioned

attribute “dried tobacco leaves” with a score of 67, cluster scoring somewhat higher for “vanilla” with a score of 36 and for “cocoa/dark chocolate” with a score of 27 than other clusters). Fig extract “Max” (0.15%) was allocated to cluster 12 (most frequently mentioned attribute “dried tobacco leaves” with a score of 90, cluster scoring somewhat higher for “dried tobacco leaves” and for “licorice” (score of 28) than other clusters). Both fig extract test products were rated as “Out” by none of the consumers in the “In/Out” test and were therefore not further assessed in the CATA test.

There are fundamental methodological flaws with using the “In/Out” test of two products with fig concentrations to make the conclusive statement that fig additive does not result in a characterizing flavor. Additionally, other vital information is missing from the industry report that could be a determining factor in whether or not fig would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which fig was incorporated in the tobacco product, as well the quantity of the remaining fig. Furthermore, the methods used to select sensory assessors are not considered valid. The reported study used assessors (adult smokers) who were likely to have lower sensitivity to the odor of fig in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits (i.e. young, non-smokers). In addition, the screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor, and because the selection criteria identified in advance of testing were often not applied.

Evaluation by the review panel: Even in the absence of a characterizing flavor, the addition of fig can increase the attractiveness of cigarettes and RYO by modifying the perceived flavor, taste or odor of the final product. The industry report does not contain any information about the potential increase of attractiveness on cigarettes and RYO by the addition of fig. The impact of fig on tobacco flavor is likely to have been underestimated in the reported study.

5.4.8 Overall conclusion on additive

The industry evaluated fig juice application levels of up to 0.3%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry’s assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Fig is a non-volatile complex mixture consisting of compounds that undergo pyrolysis. Although no new pyrolysis experiments were performed, some of the listed pyrolysis products have CMR properties. The classified carcinogen furfural has been listed as pyrolysis product but was not included in the chemical comparative analysis. Thus, an increasing effect on CMR properties of the mainstream smoke cannot be ruled out. Inhalation toxicity was not evaluated in the industry report. Thus, given the application level of up to 0.3% and based on the presented data, it cannot be concluded that use of fig as an additive in cigarettes and RYO tobacco is without concern regarding toxicity. Influence on inhalation facilitation and addictiveness have not been assessed adequately. Although some pyrolysis products of fig are known precursors for MAO inhibitors, no increase was found for analyzed aldehydes at tested levels. Possible decrease of smoke pH due to addition of fig that would make inhalation more comfortable has also not been ruled out. Influence on inhalation facilitation remains unclear. As fig is used to enhance tobacco taste, there is still a concern that it increases the attractiveness of tobacco products, by attenuating bitterness and harshness of tobacco.

The main concerns of the review panel with regards to using fig as an additive in cigarettes and roll your own tobacco is the carcinogenicity of pyrolysis products (furfural) and its flavoring properties

that enhance attractiveness.

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5.5 Report of Geraniol

5.5.1 Abstract

Geraniol is used as a flavoring agent in food and as a fragrance in cosmetics. It is applied in commercial products as a tobacco additive in amounts up to 0.03%. Application levels in the industry report were 0.015% (Low), 0.030% (Max), and 0.045% (Max-plus). Geraniol is a volatile compound with a transfer rate of 7-8%. During pyrolysis, 85.6% of the substance stayed intact and reported pyrolysis products do not have CMR properties. The industry concluded in their report that application of geraniol did not result in significant effects regarding smoke chemistry, toxicity, addictiveness, inhalation facilitation or characterizing flavor. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. The main concerns of the review panel with regard to using geraniol as an additive in cigarettes and roll your own tobacco are its potential to facilitate inhalation of smoke, which can potentially increase addictiveness and exposure to toxic substances from smoke, and its flavoring properties that enhance attractiveness. The use of geraniol is already prohibited in at least one Member State (i.e. Germany) due to its potential to facilitate inhalation.

5.5.2 Background

Geraniol is the cis isomer of a terpene alcohol. It is used as flavor (FI 02.012) in food, cosmetics and other consumer goods. Further, geraniol occurs as a constituent of essential oil such as rose oil or citronella oil.

Previous assessments of scientific data on geraniol as an additive in tobacco products have identified several concerns (1, 2).

Regarding toxicity: Geraniol has a high potential for skin sensitization and is a skin and eye irritant. Sensitization in animals has been shown for oxidation products of geraniol (e.g. geranial, epoxy-geraniol, epoxy-geranial). No information on respiratory sensitization is available, although it is seen as likely by SCENIHR. A possible impurity, methyleugenol is a genotoxic carcinogen.

Regarding addictiveness: Geraniol is also a weak agonist of the menthol receptor TRPM8. As a tobacco additive, it could act in combination with menthol or other TRPM8 agonists (i.e. isopulegol, linalool) to facilitate inhalation of cigarette smoke.

Regarding characterizing flavor: Geraniol is a known flavoring agent for food. It is added to tobacco products for the same purpose.

5.5.3 Literature review

The industry report provides two literature overviews for geraniol, one regarding additive in general and a second one regarding geraniol when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4). For example, some important sources were not used (i.e. ECHA (3)). Relevant issues, such as physiological functions/properties or effects by inhalation are not sufficiently covered.

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report for geraniol.

5.5.4 Chemistry and pyrolysis products

The report provides a brief description of geraniol as an additive. In general, synthetic geraniol (CAS 106-24-1) with a purity of 97% is used as tobacco additive. The composition of the remaining 3% is not provided, however the report states that the supplier provided a confirmation that the compound was free of methyl eugenol. The submitted data cover information on the manufacturer and compliance with EU regulations concerning flavorings and food ingredients with flavoring properties.

Application levels: The geraniol levels applied in test cigarettes varied between 0.015 and 0.045 % (Low 0.015 %; Max 0.03 %; Max-plus 0.045 %). In comparison, the maximum application level in cigarettes and roll-your-own (RYO) tobacco reported by the industry is 0.03 %. Actually achieved concentrations after application of geraniol were determined as 0.016%, 0.025%, and 0.053% for the Low, Max, and Max-plus application levels, respectively.

Transfer of geraniol into mainstream smoke: There are no published data on the transfer of geraniol yet. The industry performed new tests for the three application levels, demonstrating transfer rates of 7-8 %.

Pyrolysis experiments: The industry did not perform new pyrolysis studies. Previous studies confirmed that 85.6 % of geraniol that transferred into the MSS did not pyrolyze. Pyrolysis products, as identified by Baker and Bishop (4), included citral (4.6 %), beta-myrcene (3 %); ocimene (1.8 %); neryl acetate (1.3 %); alloöcimene (0.7 %), menthatriene (0.5 %), limonene (0.4 %), as well as 20 other minor products. Although some of these compounds have previously been described as tobacco smoke constituents (i.e. alloöcimene (5)), are used as flavors (i.e. ocimene) or are known constituents in other essential oils (i.e. neryl acetate, beta-myrcene), no assessments of their properties were provided by the industry.

Chemical analysis of mainstream smoke: For comparative testing of main stream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.015- 0.045 %).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, is included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In

this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

In the industry experiments, the comparative testing approach showed some differences between the test cigarettes and the additive free reference product. The difference in levels of acetaldehyde, acetone, acrolein, butyraldehyde, crotonaldehyde, formaldehyde, propionaldehyde, NNK, NO, NOx and water exceeded the variability of the 3R4F monitor cigarette. However, the statistical analysis performed by the industry did not result in any overall statistically significant increase or decrease of any of the tested chemical compounds.

Since it is known that aldehydes may inhibit monoamine oxidases (MAOI) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). As the data was of poor quality, it was difficult to draw conclusions on the influence of geraniol. Although there were increased levels of carbonyl formation, the review panel concluded that with the provided data it was not possible to conclude that these were attributable to geraniol, as the compound levels did not increase with increasing levels of geraniol in the test cigarette.

Evaluation by the review panel: The submitted report on geraniol examines the transfer, pyrolysis, as well as the levels of harmful and potentially harmful constituents in cigarette smoke. However, no new pyrolysis experiments were performed and there are limitations in the comparative testing approach. Geraniol is however applied in very low levels (< 0.05%), with a low transfer rate into mainstream smoke (7-8%). In addition, pyrolysis experiments demonstrated that less than 15% of geraniol is pyrolyzed. The industry concluded that no statistically significant and consistent additive-level related increases or decreases were recorded for any smoke constituent. Although, there were several limitations in the approach and methodology, an influence on smoke chemistry from the application of geraniol as an additive in cigarettes and RYO tobacco cannot be ruled out, but is unlikely given its low application level, decomposition during the pyrolysis phase and the level of changes in emissions in the chemical comparative experiments.

5.5.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – PARTLY ASSESSSED

As discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relatively extensive evaluation of the toxicity of geraniol when ingested. Nevertheless, the literature search is biased, there is no data on *in silico* tests, and no carcinogenicity, reproductive and development studies have been conducted. The report provided by the industry is almost exclusively based on conclusions of the EFSA and JECFA evaluations (6, 7). Based on these, the industry considers geraniol to be non-genotoxic, non-mutagenic, and non-carcinogenic.

However, in a search performed by the review panel, a geraniol ECHA registration dossier was identified, as well as two reproductive toxicity studies and a developmental toxicity study that

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

highlighted toxic effects. These studies should have been mentioned and discussed in the report. Also, it should be noted that geraniol has been assessed recently by ECHA and classified as Skin Sens 1 (3).

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

The inhalation toxicity of geraniol is not evaluated in the industry report. Based on a search performed by the review panel, literature on geraniol toxicity by inhalation seems limited and focused on its use as a fragrance with other substances. One sub-chronic study is available in the ECHA registration dossier. It is a study in rats exposed to a mixture of fragrances containing geraniol, for 6 or 13 weeks, 4 hours per day, 5 days a week. No abnormalities in any parameter were observed when geraniol was used in a fragrance mixture (8). However, one publication suggested that inhalation of geraniol can induce hepatotoxic effects in rat (9). As data on inhalation are rare, all available evidence needs to be assessed, even if these effects would not be relevant in the context of smoking. It is a shortcoming of the report that neither inhalation toxicity, nor physiological effects are sufficiently covered by the industry report.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The pyrolysis products identified in the report were not evaluated in terms of oral nor inhalation toxicity. Moreover, the identification of pyrolysis products was based on literature applying the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of geraniol were not determined. Based on an independent assessment by the review panel it was concluded that one of the reported pyrolysis products (betamyrcene) is classified as possibly carcinogenic to humans (2B) by the International Agency for Research on Cancer (IARC) (10).

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

For comparative testing, the report includes both a literature review and new studies. Based on the literature, the industry report concluded that *in vitro* and *in vivo* studies did not indicate an increase in toxicity due to application of geraniol as a tobacco additive. Similarly, the industry did not identify significant effects of geraniol in their comparative experiments, and concluded that at inclusion of 0.015 – 0.045% of geraniol in tobacco does not increase the CMR properties of the mainstream smoke.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies were not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 0.015 – 0.045% of geraniol in tobacco does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. The conclusion of the review panel is that the current report is not sufficient to reach a conclusion regarding the toxicity of geraniol when used as a tobacco additive. There were several methodological limitations in the comparative testing approach, and no data was presented on the inhalation toxicity of geraniol and its pyrolysis products.

In spite of these limitations in the approach and methodology, a contribution to increased toxicity and CMR properties upon application of geraniol in tested levels is unlikely, given its low application level, transfer rate, pyrolysis rate and changes in mainstream smoke composition, but cannot be ruled out.

5.5.6 Addictiveness, inhalation facilitation and nicotine uptake

Concerns to be addressed:

Addictiveness: No concerns were identified by SCENIHR (opinion 1) or other reviewed independent sources regarding possible addictive effects of geraniol or its pyrolysis products.

Inhalation facilitation: Geraniol is a weak agonist of the menthol receptor TRPM8 (1) that can induce a sensation of pleasant cooling and was shown to facilitate inhalation of irritating gases (also see report on menthol). Activation of TRPM8 is an intrinsic physiological property of geraniol, although this activity is comparatively weak in relation to menthol (1). Further, it can suppress mediators of pulmonary inflammation (11) and alleviate asthma in an animal model (12). The industry report notes that it has not yet been explored whether geraniol can reduce the harshness of smoke, and thus, promote inhalation of irritating aerosols, but did not present any new assessments.

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of geraniol, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added geraniol to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of geraniol on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from geraniol. There were also no studies reported assessing the effects of geraniol on nicotine bio-availability and clinical markers of addiction, such as craving, withdrawal symptoms or dependence scores.*

Evaluation by the review panel: The industry concludes that there was no effect of adding geraniol at tested levels on inhalation facilitation and nicotine uptake. In the review panels opinion, these data are limited and do not address the previously identified concern of TRPM8 activation. The fact that independent data show that geraniol is an agonist of TRPM8 receptor indicates that there may be an influence on inhalation facilitation due to addition of geraniol to tobacco.

5.5.7 Characterizing flavor

Although geraniol is a known flavoring agent, it is not possible to conclude whether the additive geraniol can be responsible for introducing a characterizing flavor or odor to tobacco products, on the basis of the evidence presented in the industry report. This is primarily due to the many uncertainties relating to how the evaluation was conducted and how the data might be interpreted. Specifically, the report indicates that overall 14 attributes were identified by three trained panelists, in which both test products containing geraniol were described by the panelists as having a “lemon” note. Cluster analysis using 15 consumers screened for their sensory ability: the two test products (geraniol Max 0.03%, geraniol Low 0.015%) containing different levels of geraniol were allocated to cluster 2 (most frequently mentioned attribute “dried tobacco leaves” with a score of 56, cluster scoring somewhat higher for “dried fruits”, with a score of 44, and “lemon”, with a score of 38, than other clusters). Both Geraniol Test products were rated with an “out” score of 3 (out of 10) in the “in/out” test. As the threshold of 6 “out” was not reached, the test products were not further assessed in the CATA test.

There are fundamental methodological flaws with using the “In/Out” test of two products with geraniol concentrations to make the conclusive statement that geraniol additive does not result in a characterizing flavor. Additionally, other vital information is missing from the industry report that could be a determining factor in whether or not geraniol would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which geraniol was incorporated in the tobacco product,

as well the quantity of the remaining geraniol. Furthermore, the methods used to select sensory assessors are not considered valid. The reported study used assessors (adult smokers) who were likely to have lower sensitivity to the odor of geraniol in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits (i.e. young, non-smokers). In addition, the screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor, and because the selection criteria identified in advance of testing were often not applied.

Evaluation by the review panel: As a result of the described methodological limitations, and taking into consideration the wide use of geraniol as flavor agent in other products, the impact of geraniol on tobacco flavor is likely to have been underestimated in the reported study. However, even in the absence of characterizing flavors, the addition of geraniol can increase the attractiveness of cigarettes and RYO by modifying the perceived flavor, taste or odor of the tobacco product. The industry report does not address the potential increase of attractiveness of cigarettes and RYO by the addition of geraniol.

5.5.8 Additional information based on independent data sources – as retrieved by WP 9 participants

Assessments based on thresholds of toxicological concern (TTC) are frequently used to assess flavors. EFSA had applied a TTC according to Cramer class (6). Consequently, a daily oral exposure of 1.8 mg (26 µg per kg bw) would be of no concern. Based on 700 mg tobacco per cigarette, content per cigarette is estimated as 0.21 mg geraniol. Regarding an estimated transfer of 10% into MSS, exposure levels are set to 0.021 mg per cigarette or 0.42 mg per day (1 package of cigarettes). This is well below the TTC for Cramer class I. However, caution has to be taken, if conclusions were extended to the inhalation route. According to the JECFA, an ADI of 0.5 mg per kg bw can be applied to geraniol (7). As the estimated exposure by smoking would be app. 83-fold below the ADI-level (70 kg = 35 mg per day, no relevant toxicological risks are expected. This conclusion can also be extended to putative pyrolysis products, since 85.6% of geraniol remains intact. Cramer class I can also be applied for citral (6), as major pyrolysis product (4.6%). However, it remains a shortcoming of the industry report that no assessments have been performed for other identified products.

5.5.9 Overall conclusion on additive

The industry evaluated geraniol application levels of up to 0.045%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted report. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Geraniol is applied at low levels in tobacco (<0.05%), and is a volatile compound with low transfer rate (7-8%) that mostly stays intact during pyrolysis (85.6%). Although no new pyrolysis experiments were performed, one of the reported pyrolysis products is classified as possibly carcinogenic to humans (2B) by IARC (10). Also, inhalation toxicity is not evaluated in the industry report. Nevertheless, the review panel concluded that the low application level, transfer rate and pyrolysis rate suggested that a contribution to increased toxicity and CMR properties is unlikely, but cannot be ruled out.

Independent literature shows that geraniol activates the "cold and menthol receptor" TRPM8. This can potentially lead to facilitation of inhalation and a subsequent increase of nicotine uptake, and is thus a reason for concern. Importantly, this is an intrinsic property of geraniol and does not comply

with Article 7 of the TPD, even if application levels in tobacco might not induce measurable effects. Although the potency of geraniol is relatively low, in combination with other agonists of the cooling receptor TRPM8, it could contribute to relevant menthol-like effects regarding inhalation. In addition, geraniol is known to be a flavoring additive. Addition of flavorings makes tobacco products attractive and palatable, and is therefore cause for concern.

The main concerns of the review panel with regard to using geraniol as an additive in cigarettes and roll your own tobacco are its potential to facilitate inhalation of smoke, which can potentially increase addictiveness and exposure to toxic substances from smoke, and its flavoring properties that enhance attractiveness. The use of geraniol is already prohibited in at least one Member State (i.e. Germany) due to its potential to facilitate inhalation.

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5.6 Report of Glycerol

5.6.1 Abstract

Glycerol is a sweet-tasting, odorless and colorless liquid naturally present in animal and vegetable fat. It has been widely used for years in many industrial consumer products such as detergents, cosmetics, medicines, food and drinks, resins, etc. In tobacco, glycerol is used as a humectant to trap and keep tobacco moist, in application levels of up to 5%. In the industry report three levels are tested, 2.5% (Low), 5% (Max) and 6% (Max-plus). Glycerol has a low volatility in ambient conditions and the transfer rate is 4.5%. 99.8% of glycerol stayed intact during pyrolysis. It has no CMR properties. The industry concluded in their report that application of glycerol did not result in significant effects regarding toxicity, addictiveness, inhalation facilitation or characterizing flavor. Concerning smoke chemistry, a decrease of NAB, catechol, hydroquinone, *m+p*-cresol, o-cresol, phenol, and quinoline, and an increase of glycerol was described as a significant "overall effect" by the industry. The review panel concluded that the low quality of provided data (see Chapter 4) does not allow complete interpretation of comparative emission testing. Also, the chemical and toxicological comparative analysis lacked an appropriate control; a test cigarette without a humectant was used as a reference resulting in altered combustion conditions likely to affect the levels of toxicants in mainstream smoke. The assessments of toxicity, addictiveness, inhalation facilitation, and characterizing flavor were considered to be insufficient. The main concerns of the review panel with regards to the assessment of glycerol as an additive in cigarettes and roll your own tobacco are the yet unclarified influence on smoke chemistry, the insufficient data to rule out toxicological concerns and the possible impact on the attractiveness.

5.6.2 Background

Glycerol is used as a humectant; it helps to keep tobacco moist and is added during the casing process. It can represent up to 4.5% of the total weight, making it one of the most predominant additives in tobacco. Previous assessments of scientific data on propylene glycol as an additive in tobacco products have identified several concerns (1, 2).

Regarding toxicity: The main concern regarding glycerol is the formation of acrolein. Acrolein is a toxic compound which, when inhaled, can irritate the respiratory tract epithelium besides its other toxic effects including DNA and protein adduction, oxidative stress, and immune dysfunction.

Regarding attractiveness: As humectants are added to trap water, it can positively influence the attractiveness by improving the palatability of cigarettes, making them more appealing and easier to smoke. Increasing attractiveness can lead to an increase in tobacco consumption, and thus to greater exposure to toxic compounds.

Regarding addictiveness and characterizing flavor: There are currently no data to suggest an addictive effect of glycerol, and it is not expected to have a characterizing flavor.

5.6.3 Literature review

The industry report provides two literature overviews for glycerol, one regarding additive in general and a second one regarding glycerol when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4).

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major

limitation of the industry report.

5.6.4 Chemistry and Pyrolysis products

Glycerol (CAS 56-81-5) is used as humectant in tobacco to adjust moisture and hygroscopic properties of tobacco.

Application levels: Glycerol levels applied in test cigarettes varied between 2.5% and 6 % (Low 2.5 %; Max, 5.0 %; Max-plus 6 %).

Transfer of glycerol into mainstream smoke: The industry performed new tests for the three application levels, demonstrating transfer rates of 4.2 – 4.5 %.

Pyrolysis experiments: The industry did not perform new pyrolysis studies. Baker and Bishop found that glycerol stayed intact to 99.8 %. The remaining 0.2% were two unidentified products.

Chemical analysis of mainstream smoke: For comparative testing of main stream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (2.5 - 6 %).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, is included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F) . In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

Further, the glycerol-free control cigarettes lack glycerol, but also any other humectant. This might affect moisture, burning properties and the composition of tar, perhaps leading to modified toxicant levels not attributable to specific properties of glycerol. Not even a "Low" application level of propylene glycol was applied as an alternative humectant, which is regarded as a notable methodological mistake. The review panel questions, whether the "additive-free" reference cigarette can be regarded as an appropriate control. Such a product represents a faulty manufactured cigarette that is lacking an important technical feature, and is not likely to be placed on the market. In fact, levels of benzo[a] pyrene that might be regarded as combustion marker decrease in relation with glycerol, and are highest in the "additive free" reference product. This indicates that combustion conditions are different with lower application levels of glycerol. However, there are no trends for other relevant compounds, as for example carbonyls. Humectants are technical compounds that are required to manufacture tobacco products. Therefore industry should have analyzed whether glycerol can be regarded as comparatively safe option or whether other applicable humectants bear lower risks. The

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

toxic properties of glycerol should have been studied and summarized in relation to propylene glycol and other compounds that are used as humectant in tobacco. In addition, information is missing on the glycerol content of the 3R4F reference cigarette that was used as a basis for statistical evaluation, which is roughly similar to the "Low" (2.5%) test cigarette (3).

Application of glycerol at "Max" and/or "Max-plus" levels has resulted in decreases of benzo[a] pyrene, N'-nitrosonornicotine (NNN), N'-nitrosoanabasine (NAB), catechol, hydroquinone, m+p-cresol, o-cresol, phenol, pyridine, and quinoline that exceeded the variability of the 3R4F monitor cigarette. Some of these effects were regarded as a statistically significant "overall effect". Increase of ammonia at "Max-plus" application level and increase of water at all application levels exceeded variability of the 3R4F reference cigarette. Interestingly, both effects were not regarded as a statistically significant "overall effect".

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). No increase of carbonyls was found at tested levels. Levels of acrolein in mainstream smoke, which have been re-evaluated as well, were not found to be increased compared to additive-free reference cigarette.

Evaluation by the review panel: Taken together, there are conceptual- and technical shortcomings in the data on smoke chemistry. To address these issues appropriately, industry should comprehensively summarize effect of humectants on toxicant levels and on other relevant properties of cigarettes, besides providing data on specific compounds. Shortcomings in the industry's approach and methodology to assess smoke chemistry limit the usefulness of provided data. Although the data provided by the industry do not suggest an increase of toxic compounds due to combustion of glycerol, the provided experiments are not sufficient to determine whether propylene glycol affects smoke chemistry.

5.6. Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – ASSESSSED

As discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relatively extensive evaluation of the toxicity of glycerol when ingested. The report provided by the industry is almost exclusively based on the opinion of the EFSA ANS panel (4) and the OECD SIDS panel (5) and concludes that ingestion of glycerol has no carcinogenic, mutagenic/genotoxic or reprotoxic properties. The EFSA opinion concludes to a very low acute oral toxicity potential. However, it also highlights the existence of toxicity for the gastrointestinal tract when administrated continuously via the diet. In the EFSA opinion on the re-evaluation of glycerol as a food additive, in addition to the formation of acrolein from heated glycerol, it is clearly stated that acrolein and other impurities of genotoxic compounds (glycidol, epichlorohydrin) can be formed during the manufacturing process and consequently be present in the product used as an additive. It is therefore of paramount importance to consider very carefully the chemical composition of the glycerol used as additive in tobacco products.

It was recalled in the industry report that glycerol is widely used in many consumer goods, such as foods, cosmetics, personal care products and in pharmaceuticals, so there is a long history of consumer exposure to glycerol. Nevertheless, exposure from the diet is completely different from exposure by inhalation, and such effects are not representative for the inhalation route. Therefore, it is not relevant to use the assessment that was conducted for the use of glycerol as a food additive to evaluate its toxicity when included in tobacco products.

B. Evaluation of **additive itself (inhalation)** – PARTLY ASSESSED

The inhalation toxicity of glycerol is briefly evaluated in the industry report. This section consists of two *in vivo* studies on chronic toxicity only. These are relevant studies, and although CMR effects are not expected, they are not assessed. Also, information regarding metabolite formation and their possible toxicity is lacking. Glycerol has a low volatility, but a transfer mainly in its unburnt form to mainstream smoke is expected. No effects were found at the highest dose of glycerol (respectively 3910 mg/m^3 and 662 mg/m^3) in the subchronic and chronic studies in rats quoted in the industry report (3,4) and there is no study in the literature presented by industry that show an acute or chronic toxicity by the inhalation route.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The industry report does not evaluate the toxicity of pyrolysis products. They stated that glycerol is expected to transfer intact in smoke, based on previous studies (4-7). Transfer rates between 4-8 % have been determined. Only two unidentified products where measured in the study from Baker *et al.* (8). However, as the identification of pyrolysis products was based on the Hoffmann list it is possible that some potentially toxic compounds resulting from pyrolysis of glycerol were not identified.

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

For comparative testing, the report includes both a literature review for *in vivo* studies and new *in vitro* tests. The included *in vivo* tests showed a statistical difference for one parameter, namely a 13 – 15% increase of carboxyhemoglobin (9), no other biological effects were noted during exposure to glycerol cigarette smoke compared to control groups. None of the mentioned studies in the industry report suggested that glycerol in mainstream smoke could have CMR properties or other toxic effects.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). Similarly, the lack of an appropriate control also affects the interpretation of the toxicity comparative testing. In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 2.5-6 % glycerol does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, the review panel concludes that the presented toxicological assessment of glycerol is insufficient. There were several methodological limitations in the comparative testing approach, including the lack of inclusion of an appropriate control. Additionally, pyrolysis products that are formed at low amounts (0.2%) were not identified. Finally, the previously identified concern regarding increased exposure to acrolein was not addressed in the industry report. Thus, an influence on toxicity upon application of glycerol as an additive in cigarettes and RYO tobacco cannot be ruled out.

5.6.6 Addictiveness, inhalation facilitation and nicotine uptake

Concerns to be addressed:

Addictiveness: No concerns were previously identified regarding possible addictive effects of glycerol or its pyrolysis products.

Inhalation facilitation: Glycerol is added to tobacco as a humectant, to keep moisture in tobacco and prevent it from drying out. This humidification reduces the harshness of smoke and may thereby facilitate inhalation (2).

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of glycerol, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added glycerol to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of glycerol on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for glycerol or any of its metabolites. There were also no studies reported assessing the effects of glycerol on nicotine bio-availability and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores.*

Evaluation by the review panel: The provided data show no effect of adding glycerol on inhalation facilitation and nicotine uptake, but these data are limited and do not address the previously identified concern regarding humidification. Altogether, there is insufficient evidence to rule out an influence on inhalation facilitation upon application of glycerol as an additive in cigarettes and RYO tobacco.

5.6.7 Characterizing flavor

Whether the additive glycerol can be responsible for introducing a characterizing flavor or odor to tobacco products has not been addressed in the industry report. However, as glycerol is not known to be a flavoring compound, no such effect is expected. However, possible reactions of glycerol combustion with other compounds found in the smoke cannot be excluded. Glycerol has a ”sweet” taste that can mask the harshness of smoke and thus increase the attractiveness of the tobacco products (1). This is not assessed by the industry.

5.6.8 Overall conclusion on additive

The industry evaluated glycerol application levels of up to 6 %, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. In spite of the before mentioned limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Glycerol is a non-volatile humectant that transfers mostly intact. Glycerol has a large influence on smoke chemistry that has not been clarified by industry properly. As humectants are technically necessary compounds, influence of glycerol on toxicity should have been assessed in comparison to other humectants that could be applied instead of glycerol. Although pyrolysis products are formed at a very low extent (0.2%), they have not been identified. Thus, their contribution to toxicity cannot be evaluated. Inhalation toxicity was assessed by the industry. However, evidence is not sufficient to rule out any increase in toxicity. Influence on inhalation facilitation and addictiveness has not been sufficiently assessed. Glycerol is a humectant that keeps the tobacco moist and thus increases palatability of the product. No assessment has been performed regarding influence on attractiveness.

The main concerns of the review panel with regards to the assessment of glycerol as an additive in cigarettes and roll your own tobacco are the yet unclarified influence on smoke chemistry, the insufficient data to rule out toxicological concerns with certainty and the possible impact on the attractiveness.

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5.7 Report of Guaiacol

5.7.1 Abstract

Guaiacol is a naturally occurring organic compound that is widely used in food and other consumer products such as cosmetics. In tobacco products, it is added in amounts up to 0.001%. In the industry report three levels are tested, 0.0005% (Low), 0.001% (Max) and 0.0015% (Max-plus). Guaiacol is a volatile compound with an unknown transfer rate, but transfer of supplemented guaiacol seems to be negligible compared to guaiacol formation by tobacco lignin pyrolysis. Guaiacol stays mainly intact during pyrolysis (92.5 %), with one main pyrolysis product, guaiacol acetate (6.3 %), that is not classified to have CMR properties. The industry concluded in their report that application of guaiacol did not result in significant effects regarding smoke chemistry, toxicity, addictiveness, inhalation facilitation or characterizing flavor. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. Although guaiacol is classified as an irritant, the review panel concludes that an influence on smoke chemistry upon application of guaiacol as an additive in cigarettes and RYO tobacco, is unlikely given its low application level and its higher occurrence in burnt tobacco, but cannot be ruled out. The main concerns of the review

panel with regard to using guaiacol as an additive in cigarettes and roll your own tobacco are its potential anesthetic effects that might facilitate inhalation and its flavoring properties that enhance attractiveness.

5.7.2 Background

Guaiacol (2-methoxyphenol, catechol monomethyl ether) is a naturally occurring organic compound and a flavoring agent for food. Although it is biosynthesized by a variety of organisms, this yellowish aromatic oil is usually derived from guaiacum or wood creosote. It is added to tobacco products for flavoring. Previous assessments of scientific data on guaiacol as an additive in tobacco products have identified several concerns (1).

Regarding toxicity: Guaiacol is a severe eye irritant, a skin irritant, and is also reported to be a respiratory tract irritant. Other toxicological information on repeated exposure is scarce.

Regarding addictiveness: It is used as a local anesthetic, and can enhance smoke inhalation, thus potentially contributing to addictiveness by increasing nicotine intoxication levels.

Regarding characterizing flavor: It is added to tobacco products for flavoring, thereby potentially contributing to attractiveness. More data are needed on the amount of guaiacol that imparts a noticeable flavor to tobacco.

5.7.3 Literature review

The industry report provides two literature overviews for guaiacol, one regarding the additive in general and a second one regarding guaiacol when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4). For example, some important sources were not used, and relevant issues, such as physiological functions/properties or effects by inhalation are not sufficiently covered.

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.7.4 Chemistry and pyrolysis products

The report provides a brief description of guaiacol (CAS 90-5-1), summarizing chemical characteristics and lot properties. The submitted data cover information on the manufacturer and compliance with applicable EU food flavoring legislation.

Application levels: The guaiacol levels applied in test cigarettes varied between 0.0005% and 0.015 % (target concentrations Low 0.0005 %, Max 0.001 %, Max-plus 0.0015 %; concentrations actually achieved were 0.00056%, 0.00088%, and 0.00156%, respectively).

Transfer of guaiacol into mainstream smoke: A mean yield of 1.7µg/cig was found for the Low (0.0005 %), Max (0.001 %) and Max-plus (0.0015 %) test cigarette in the provided transfer study. A slightly lower yield of 1.5 µg/cig was found for the additive free reference samples. Submitted data demonstrate that guaiacol occurs in MSS of reference cigarettes that did not contain this compound as an additive. However, the amount of guaiacol in the MSS increased about 13% in average after addition of 0.0005%-0.0015%. These data suggest that even though guaiacol is present in MSS of additive-free cigarettes, application of guaiacol as an additive leads to a further increase.

Pyrolysis experiments: Pyrolysis experiments performed with lignin report the formation of many guaiacol derivatives besides guaiacol itself. However, guaiacol is transferred largely intact into the smoke. The industry report takes reference to published pyrolysis studies, demonstrating that more than 90 % of guaiacol is transferred as intact molecules into the MSS (2). This finding was also confirmed in other studies (3, 4). Baker and Bishop further identified guaiacol acetate (6.3 %), indanone (0.7 %), dimethoxybenzene (0.3 %) and chinnoline (0.2 %) as pyrolysis products of guaiacol (2). No supplementary pyrolysis tests were performed to confirm these results.

Chemical analysis of mainstream smoke: For comparative testing of main stream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.0005 - 0.0015 %).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, is included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

The presented data on smoke chemistry from test cigarettes containing guaiacol show a comparatively high variability when compared with data for other additives, including cocoa, menthol or fig extract. Notably, the relative differences between test cigarettes containing guaiacol and additive-free reference cigarettes did exceed the variability of 3R4F monitor cigarettes for several compounds, including acetaldehyde, acetone, acrolein, butyraldehyde, crotonaldehyde, formaldehyde, propionaldehyde, NNK and water. In fact, low dosed test cigarettes (min-group) generated higher levels of all tested carbonylic compounds, as compared to Max-plus samples and additive free reference products. Further, carbonyl analysis of one test series (guaiacol, geraniol, guar gum, maltol, propylene glycol, sorbitol) resulted in lower carbonyl yields and higher standard deviation for the same additive-free cigarette, than in the other test series (carob bean, cocoa, fenugreek, fig, glycerol, licorice, menthol) (see Chapter 4). Standard deviations were also high for the tested cigarettes in the first series. It is regarded as shortcoming that no attempts were made to explain these findings. However, a literature study showed that inclusion of 0.00001 to 0.003% guaiacol in test cigarettes resulted in statistically significant increase of several compounds, such as 4-aminobiphenyl, eugenol, toluene, styrene, acrolein and propionaldehyde (5).

The industry claims that guaiacol does not affect the composition of MSS for three reasons. First, it was applied in the very low application level of only 0.001 % that they reported as the Quantity Not Exceeded level used by the participating companies, i.e., the highest concentration used in any of their products. Second, they point out that there are comparable guaiacol yields in smoke from additive-free cigarettes, as this compound is endogenously generated by tobacco lignin pyrolysis.

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

And third, the observed increases of some smoke components do not show an additive-related dose response and are usually highest in the low dosed samples.

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for aldehydes presented by the industry (see Section 3.4 and Annex II). As the data was of poor quality, it was difficult to draw conclusions on the influence of guaiacol. Although there were increased levels of carbonyl formation, the review panel concluded that these cannot be attributable to guaiacol with certainty, as the compound levels did not increase with increasing levels of guaiacol in the test cigarette (see discussion above).

Evaluation by the review panel: The submitted report on guaiacol provides data regarding the transfer, pyrolysis, as well as the levels of harmful and potentially harmful constituents in cigarette smoke. However, no new pyrolysis experiments were performed and there are several limitations in the comparative testing approach. Guaiacol is however applied in very low levels (Max-plus 0.0015%) and is generated by pyrolysis of lignin from tobacco. Even if most of the guaiacol in mainstream smoke seems to stem from the tobacco pyrolysis, the addition of guaiacol in the cigarettes in very low levels leads to a further increase of guaiacol in MSS. The industry concluded that no statistically significant and consistent additive-level related increases or decreases were recorded for any smoke constituent. Although, there were limitations in the approach and methodology, the members of the review panel conclude that an influence on smoke chemistry of the analyzed emitted compounds upon application of guaiacol as an additive in cigarettes and RYO tobacco, is unlikely given its low application level and its higher occurrence in burnt tobacco, but cannot be ruled out. Similarly, an increase of other MSS compounds due to addition of guaiacol can also not be excluded. Furthermore, as discussed above, data indicate that some technical issues have not been sufficiently addressed by industry. Although comparative testing is a valuable approach, some refinements might be required. This might include additional controls. Further criteria (as for example comparable yield of water per cigarette) should be defined to ascertain comparability in relation to tested additives.

5.7.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation, these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – ASSESSSED

As discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relative extensive evaluation of the toxicity of guaiacol when ingested.

An LD50 of 621 mg/kg bw was identified in both gender groups of Swiss mice (male/female) given a single oral dose of guaiacol. Data on subchronic, chronic, reproductive, and developmental toxicity for guaiacol are lacking. Absence of mutagenicity with the Ames assay, but positive genotoxicity/ sister chromatid exchanges (SCE) test in human lymphocytes *in vitro* (6) indicate a need for further *in vivo* studies. In later studies, guaiacol has been documented to give a negative result in the *in vivo* micronucleus assay (REACH registration dossier updated 6th Feb. 2018 (7)). In a carcinogenicity study (8), where 1.5% of guaiacol (corresponding to 1000 mg/k bw male rat/day) was administered in diet for 51 weeks, mild and moderate forestomach hyperplasia was found in 93.8 % of the rats that received guaiacol, but papilloma and carcinoma were not detected.

Guaiacol was given GRAS (Generally Considered As Safe) status by FEMA (1965) and is approved by the FDA for food use (21 CFR 121.1164). According to the harmonized classification and labelling approved by the European Union (Classification, Labelling and Packaging Regulation EC 1272/2008), this substance is harmful if swallowed (category code 4: H302), causes serious eye irritation, and causes skin irritation (category code: 2: H315, H319). It is considered non-mutagenic,

non-carcinogenic, and not toxic to reproduction or development under this regulation.

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

Inhalation studies for guaiacol are lacking, although it has been reported to be a respiratory tract irritant (1). Although the low exposure levels give no reason to expect that the exposure of guaiacol via inhalation itself will have toxic effects or enhance the mutagenic/carcinogenic effects of cigarette smoke, one cannot exclude the possibility that addition of guaiacol may increase the irritating effects of cigarette smoke to respiratory organs without going into a detailed examination of the specific studies.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The industry report presents studies indicating that guaiacol will not pyrolyze in a burnt cigarette but transfer into mainstream smoke mostly intact. The pyrolysis products identified in the report were not evaluated in terms of oral nor inhalation toxicity. Moreover, the identification of pyrolysis products was based on literature applying the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of guaiacol were not determined. Based on assessment by the review panel (see Annex III – "Pyrolysis product table"), it was concluded that none of the reported pyrolysis products had CMR properties, but guaiacol itself was classified as a skin and eye irritant.

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

The influence of guaiacol on mainstream smoke has been examined when added to test cigarettes as part of a mixture of ingredients. As stated in the chemistry section, the addition of guaiacol causes statistically significant increases in several compounds. The overall impact on toxicology is uncertain. The skin tumorigenicity of condensate prepared from cigarettes containing a number of additives in combination, including guaiacol at a concentration up to <0.1 ppm, was not indicative of any substantive effect, on the tumorigenicity of cigarette smoke condensate in a skin painting study, when compared to control cigarettes, without additives. The addition of guaiacol up to 12 ppm, did not affect the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes. Similar studies in other model systems are reported. The results of *in vitro* studies demonstrated that there were no statistically significant and meaningful increases for the mutagenicity, cytotoxicity and genotoxicity for any of the samples, when guaiacol was tested as a single additive, or as part of an additive mixture, up to a maximum inclusion level of 0.0015%.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation of the review panel: The industry concluded that inclusion of 0.0005 – 0.0015% of guaiacol in tobacco does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, guaiacol is found to be irritating to eye and skin and is considered harmful when ingested, but irritating effects in the respiratory tract are not addressed in the industry report. The conclusion of the review panel is that the current report is not sufficient to reach a conclusion regarding the toxicity of guaiacol when used as a tobacco additive. There were several methodological limitations in the comparative testing approach, and no data was presented on the inhalation toxicity of guaiacol and its pyrolysis products.

In spite of these limitations in the approach and methodology, the members of the review panel conclude that an increase in toxicity and CMR properties for application of guaiacol as an additive in

cigarettes and RYO tobacco is unlikely, given its low application level and its high occurrence in burnt tobacco, but cannot be ruled out. This evaluation is restricted to the use of guaiacol in cigarettes, and the outcome cannot be extrapolated to any use of guaiacol in other types tobacco products where guaiacol formation may not arise to the same extent from pyrolysis of tobacco constituents (lignin), including smokeless tobacco and e-cigarettes.

5.7.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: No concerns were identified regarding possible addictive effects of guaiacol or its pyrolysis products.

Inhalation facilitation: Its use as local anesthetic gives reason to believe guaiacol may favor smoke inhalation, and thus could indirectly contribute to addictiveness by increasing exposure to nicotine (1).

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of guaiacol, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added guaiacol to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of guaiacol on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from guaiacol. There were also no studies reported assessing the effects of guaiacol on nicotine bio-availability and clinical markers of addiction, such as craving, withdrawal symptoms or dependence scores.*

Evaluation of the review panel: The provided data show no effect of adding guaiacol on inhalation facilitation and nicotine uptake, but these data are limited and do not address the previously identified concern regarding guaiacol's anesthetic effect. Altogether, there is insufficient evidence to rule out an influence on addictiveness or inhalation facilitation upon application of guaiacol as an additive in cigarettes and RYO tobacco.

5.7.7 Characterizing flavor

Although guaiacol is a known flavoring agent, it is not possible to conclude whether the additive can be responsible for introducing a characterizing flavor or odor to tobacco products, on the basis of the evidence presented in the industry report. This is primarily due to the many uncertainties relating to how the evaluation was conducted and how the data might be interpreted.

The industry report concludes that based on the different sensory methodologies used (clustering, "In/Out" test and CATA testing), the addition of guaiacol at levels up to 0.001% to test cigarettes did not result in a characterizing flavor. Specifically, the report indicates that that both test products containing guaiacol were described by the sensory panelists (all adult smokers), as having a "smoked" note. Based on cluster analysis, the two test products containing different levels of guaiacol were allocated to cluster 5 (most frequently mentioned attribute was "smoked" with a score of 73, cluster scoring somewhat higher for "burned/smoke/ash" with a score of 42, and somewhat lower for "sweetish", with a score of 23, than other clusters). The guaiacol test products were rated with an "Out" score of 3 (Guaiacol Low, 0.0005%) and 4 (Guaiacol Max, 0.001%) (out of 10) in the "In/Out" test. As the threshold of 6 "Out" votes was not reached, the test products were not further assessed in the CATA test. The report thereby concluded that the different sensory methodologies used (clustering,

In/Out test and CATA testing) did not show a characterizing flavor of the test cigarettes containing two guaiacol concentrations. It should be noticed that the Max-plus concentration (0.0015%) is not assessed.

There are fundamental methodological flaws with using the "In/Out" test of two products with guaiacol concentrations to make the conclusive statement that guaiacol additive does not result in a characterizing flavor. Additionally, other vital information is missing from the industry report that could be a determining factor in whether or not guaiacol would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which guaiacol was incorporated in the tobacco product, as well the quantity of the remaining guaiacol. Furthermore, the methods used to select sensory assessors are not considered valid. The reported study used assessors (adult smokers) who were likely to have lower sensitivity to the odor of guaiacol in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits (i.e. young, non-smokers). In addition, the screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor, and because the selection criteria identified in advance of testing were often not applied.

Evaluation by the review panel: As a result of the methodological shortcomings, the impact of guaiacol on tobacco flavor is likely to have been underestimated in the reported study. The fact that guaiacol is added to unburnt tobacco, even though it is also formed during combustion, implicates that it may be added to contribute to a (characterizing) flavor or odor or taste. Nevertheless, even in the absence of characterizing flavors, the addition of guaiacol can increase the attractiveness of cigarettes and RYO by modifying the perceived flavor, taste or odor of the tobacco product. The industry report does not address the potential increase of attractiveness of cigarettes and RYO by the addition of guaiacol.

5.7.8 Overall conclusion on additive

The industry evaluated guaiacol application levels of up to 0.015%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Guaiacol is applied in low levels in tobacco (<0.0015%), and is a volatile compound with an undefined transfer rate that mostly stays intact during pyrolysis (>90%). It is also generated by pyrolysis of lignin from tobacco. Thus, most guaiacol in mainstream smoke stems from the tobacco pyrolysis rather than supplementation of an additive. Addition of guaiacol leads to a further increase of guaiacol concentration in MSS. Different application levels resulted in high variabilities of water emission, indicating either technical difficulties or an effect on chemical decomposition processes. This has not been clarified by the industry. None of the reported pyrolysis products are classified as compounds with CMR properties. However, new experiments have not been conducted. Also, inhalation toxicity was not evaluated in the industry report and possible contribution to toxicity due to inhalation irritation was not addressed. Nevertheless, the review panel concluded that the low application level and its high occurrence in burnt tobacco from pyrolysis of lignin suggest that a contribution to toxicity upon addition of guaiacol as an additive in cigarettes and RYO tobacco is unlikely, but cannot be ruled out. This conclusion does not apply to other types of tobacco products, including smokeless tobacco and e-cigarettes. Anesthetic effects of guaiacol have not been addressed and the impact on tobacco flavor is likely to have been underestimated in the reported study. Thus, contribution to inhalation facilitation has not been ruled out and there is still a major

concern that it will increase the attractiveness of tobacco products, by attenuating bitterness and harshness of tobacco.

The main concerns of the review panel with regard to using guaiacol as an additive in cigarettes and roll your own tobacco are its potential anesthetic effects that might facilitate inhalation and its flavoring properties that enhance attractiveness.

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5.8 Report of Guar gum

5.8.1 Abstract

Guar gum is made from milled guar bean seeds and consists mainly of polysaccharides such as galactomannans. It is widely used in food in functions such as thickener, stabilizer, or emulsifier. In tobacco products, guar gum is used as binder for reconstituted tobacco in amounts up to 1%. In the industry report three levels are tested, 0.5% (Low), 1% (Max) and 1.5% (Max-plus). Guar gum is a non-volatile compound and is completely pyrolyzed. Some of its pyrolysis products have CMR properties (furfural, Carc. 2; benzene, Carc. 1A, Muta 1B; toluene, Repr 2; 2-butenal, Muta 2). The industry concluded in their report that application of guar gum did not result in significant effects regarding toxicity, addictiveness and inhalation facilitation. Concerning smoke chemistry, an increase of cadmium and formaldehyde was acknowledged to be significant by the industry. Furthermore, it was not assessed whether guar gum has a characterizing flavor. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. In an independent evaluation of the chemical data, the review panel found that almost all carbonyls increased with guar gum concentrations, whereby the increase in formaldehyde was seen as significant and relevant.

Increase of other carbonyl compounds seemed to be relevant as well, but the poor quality of data (see Chapter 4) hampered data interpretation. Furthermore, a significant increase in cadmium was found. The main concerns of the review panel with regards to using guar gum as an additive in cigarettes and roll your own tobacco are the possible enhancement of addictiveness due to increased emission of aldehydes that are known MAO inhibitors, the carcinogenicity of pyrolysis products (furfural, benzene, toluene, 2-butenal), an increase of toxic compounds (especially formaldehyde and cadmium) in the emissions, and the unaddressed flavoring properties that may enhance attractiveness.

5.8.2 Background

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. It is widely used in food as stabilizer or to improve texture, consistency, softness or other product properties. Guar gum is added as a binder to reconstituted tobacco in cigarettes. Guar gum is also used to prepare the cigarette paper that wraps the tobacco. Previous assessments of scientific data on guar gum as an additive in tobacco products have identified several concerns (1-3).

Regarding toxicity: The SCHEER report describes among others that guar gum is generally regarded as safe for use in food and cosmetics. However, in the case of tobacco additive use, guar gum does not transfer intact to the mainstream smoke, but undergoes pyrolysis, giving rise to toxic/ carcinogenic compounds.

Regarding addictiveness: Aldehydes, like acetaldehyde that are generated during combustion of guar gum, enhance the addictive effects of nicotine.

Regarding characterizing flavor: Thermal degradation of sugars and carbohydrates contribute to complex aromas and several flavor compounds were reported that are due to pyrolysis reactions of guar gum. These flavor compounds singly or in combination with other smoke constituents can contribute to the attractiveness of smoking by improving smoke flavor, masking its bitter taste and reducing the harshness of smoke.

5.8.3 Literature review

The industry report provides two literature overviews for guar gum, one regarding additive in general and a second one regarding guar gum when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4). For example, some important sources were not used (i.e. ECHA). Relevant issues, such as physiological functions/properties or effects by inhalation are not sufficiently covered.

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.8.4 Chemistry and Pyrolysis products

The report, as submitted by the collaborating manufacturers, covers application levels of up to 1.5% guar gum in tobacco. Information on the supplier, lot-specification, purity and compliance with the relevant European regulation have been provided. The report includes a detailed constituent analysis.

Application levels: Guar gum levels applied in test cigarettes varied between 0.5 and 1.5 % (target

concentrations Low 0.5%; Max 1.0%; Max-plus 1.5%, with achieved levels 0.33% - 0.55%, 1.03% - 1.18%, and 1.44% - 1.57%, respectively. The applied level of guar gum in the "mix 3" cigarette was 1.0%, however the actually achieved level was not determined ("Technically not possible to detect achieved amount").

Transfer of guar gum into mainstream smoke: Guar gum is a complex solid organic additive and not expected to transfer intact into mainstream smoke (MSS). Therefore, transfer rate was not assessed by the industry.

Pyrolysis experiments: Taking reference to Baker and Bishop (4), hydroxymethylfurfural was stated as major pyrolysis product (13.4%), besides acetol (11.9%), acetic acid (9.9%), methyl pyruvate (6.1%), furfural (6.0%), cresol (0.9%), benzene (0.7%), 2-butanone (0.7%), toluene (0.5%), and 2-butenal (0.2%). Further studies that had been performed since the 1950s are cited, but not discussed in detail. No new experimental work was provided in the submitted industry report.

Chemical analysis of mainstream smoke: For comparative testing of mainstream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.5 – 1.5).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, is included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

All analyzed carbonyl compounds (acetaldehyde, acetone, acrolein, butyraldehyde, crotonaldehyde, formaldehyde, propionaldehyde) and cadmium were increased in the mainstream smoke in a dose-dependent manner. All increases exceeded the variability of the 3R4F reference cigarette at "Max-plus" application levels and for some compounds also at lower levels. In the industry report, statistically significant and consistent increases in relation to the additive have only been acknowledged for formaldehyde and cadmium. In contrast, the substantial alterations of other carbonyls, such as acetaldehyde, are hardly discussed. Guar gum consists of polysaccharides that could generate comparable pyrolysis and combustion products as sorbitol. In fact, data submitted by the industry confirm consistent and additive-related increases of carbonyls for both substances. Unfortunately, combinations of both additives have not been tested, leaving an important data gap on the interplay of tobacco additives and formation of aldehydes in cigarette smoke. This question is important for further risk assessments.

Since it is known that aldehydes, may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

compounds presented by the industry (see Section 3.4 and Annex II). Despite the poor quality of data, some information could be extracted. Almost all carbonyls increase with guar gum concentrations in an additive level related manner. The increase in formaldehyde is seen as significant and relevant. Increase of other carbonyl compounds seems to be relevant as well, but the poor quality of data (see Chapter 4) hampers data interpretation.

Notably, the observed increase of cadmium has not been sufficiently explained. The cadmium content in the guar gum sample was only 0.005mg/kg, much lower than for example in cocoa. Assessments by the industry suggest that the detected cadmium levels cannot be attributed to impurities of the guar gum sample. Neither the data on cadmium, nor the substantial variations of water and nitrogen oxides are properly explained in the report. The industry concluded based on their literature review, that no statistically relevant effects of guar gum on the smoke chemistry profile have been found in previous studies. However, several articles mentioned in the report (5-8) show that the addition of guar gum in tobacco products increases the emission of several compounds of toxicological concern, such as TPM, NH3, HCN, formaldehyde, arsenic, catechol, phenol, cresols, and others.

Evaluation by the review panel: The submitted report on guar gum examines the pyrolysis and the levels of harmful and potentially harmful constituents in cigarette smoke. However, no new pyrolysis experiments were performed and there are limitations in the comparative testing approach. Moreover, several pyrolysis products of concern were not assessed in the chemical analysis of mainstream smoke (e.g. furfural). Although there were shortcomings in the analysis of carbonyl compounds by the industry (see Chapter 4), an additive-level related increase of carbonyl compounds, especially for formaldehyde, was visible. Further, application of guar gum has led to a significant increase of cadmium. In spite of the poor quality of the provided data, the members of the review panel conclude that there may be an influence on smoke chemistry when guar gum is applied as an additive in cigarettes and RYO tobacco, given the comparatively high application level (up to 1% “Max”).

Further, guar gum could be used in combination with carbohydrates to adjust aldehyde levels in the smoke of individual cigarette brands. The interplay between guar gum, starches and other sugar supplements on aldehyde levels, as well as putative effects on addictiveness, are not sufficiently assessed.

5.8.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation. These are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – ASSESSSED

As discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. In addition, it is not known if guar gum used as a tobacco additive complies with the specifications of the food additive guar gum (E 412). However, the industry report includes a relative extensive evaluation of the oral toxicity of guar gum. No acute toxicity of guar gum was observed for doses up to 6000 and 9000 mg/ kg bw/day as assessed by JECFA and EFSA (9, 10). A NOAEL of 5000 mg/ kg bw/day was derived from the animal data (11). The available data indicated that guar gum in food was not genotoxic *in vitro* or in vivo. The available data indicated that guar gum does not have carcinogenic activity. Guar gum is not considered to be teratogenic. From a dietary combined fertility and developmental 13 weeks toxicity study in female and male Osborne–Mendel rats (Collins et al., 1987), the EFSA ANS Panel identified a NOAEL for general toxicity of 2700 mg/kg bw/day, a NOAEL of 5200 mg/kg bw/day for fertility effects and a NOAEL for developmental toxicity of 11,800 mg/kg bw/day (11).

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

Some reports indicate that guar gum may induce sensitization in some individuals in occupational

settings by inhalation (12). The tobacco industry concluded that guar gum is not associated with hazards that indicate it as a respiratory sensitizer. No "additive-only' inhalation studies were found in the industry report. According to the industry report, the pyrolysis studies have shown that guar gum undergoes pyrolysis when burnt, and components will not transfer intact, as they are of non-volatile nature.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The pyrolysis products identified in the report were not evaluated in terms of oral nor inhalation toxicity. Moreover, the identification of pyrolysis products was based on literature applying the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of guar gum were not determined. Based on assessment by the review panel (see Annex III – Pyrolysis product table) it was concluded that some of the reported pyrolysis products (furfural, Carc. 2; benzene, Carc. 1A, Muta 1B; toluene, Repr 2; 2-butenal, Muta 2) have CMR properties.

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

The results of the *in vitro* studies did not reveal statistically significant and meaningful increases of mutagenicity, cytotoxicity and genotoxicity for any of the samples (single additive or mixture; total particulate matter and gas vapor phase). The report showed results from several *in vivo* studies with guar gum added to cigarette tobacco suggesting that application of guar gum to cigarette tobacco at various levels did not discernibly alter the biological effects normally associated with mainstream cigarette smoke. However, as discussed in the Chemistry section, guar gum increases the formation of formaldehyde and cadmium. Formaldehyde is a substance classified as a CMR carcinogen cat. 1B and mutagen 2 under the EC Regulation No 1272/2008.

However, the review panel questions the validity of the industry conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 0.5-1.5% guar gum does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, in their evaluation, the review panel concluded that the presented toxicological assessment of guar gum is insufficient. There were several methodological limitations in the comparative testing approach. The chemistry results of the comparative studies showing increased emissions of several compounds of toxicological concern (such as formaldehyde and cadmium) were not followed up with toxicological assessments. Furthermore, in the comparative toxicity experiments, only CMR properties of guar gum were considered and no data was presented on the inhalation toxicity of guar gum and its pyrolysis products. Some of the reported pyrolysis products (furfural, Carc. 2; benzene, Carc. 1A, Muta 1B; toluene, Repr 2; 2-butenal, Muta 2) have CMR properties. Increase of furfural and 2-butenal levels in mainstream smoke have not been assessed in the chemical analysis of mainstream smoke. Overall, a contribution to increased CMR properties upon application of guar gum as an additive cannot be ruled out.

5.8.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: Concerns have been raised that the pyrolysis of sugars in guar gum can lead to the formation of acetaldehyde and other aldehydes, which have the potential to increase the

addictiveness of cigarette smoke via Monoamine Oxidase (MAO) inhibition (13, 14).

Inhalation facilitation: No concerns were identified regarding possible effects of guar gum or its pyrolites on inhalation facilitation. However, industry report indicates that pyrolysis of guar gum leads to the formation of acids. Decrease of smoke pH leads smokers to "increase their smoking frequency and inhale the smoke more deeply to enable a higher absorption of nicotine in the airways", as well as decreases the harshness and irritation of the smoke encouraging them "to develop a smoking habit" (15). Therefore, addition of guar gum can potentially decrease smoke pH and could then reduce harshness of smoke and facilitate inhalation.

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of guar gum, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report.*

However, no test cigarettes with the single additive guar gum were used in this study, instead guar gum was only tested in a cigarette containing a mix of additives (propylene glycol, glycerol, licorice extract powder, cocoa powder, carob bean extract, fig juice concentrate, guar gum, and fenugreek extract, geraniol, guaiacol and maltol). Outcomes of all parameters were similar between the test cigarette containing the mix of additives and the reference cigarette.

However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added guar gum to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of guar gum on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from guar gum. Specifically, none of the reported studies did asses the capacity of guar gum or its pyrolysis products on monoamine oxidase inhibition. There were also no studies reported assessing the effects of guar gum on nicotine bio-availability and clinical markers of addiction, such as craving, withdrawal symptoms or dependence scores.

Evaluation of the review panel: The fact that the provided data show increased levels of carbonyls in mainstream smoke of cigarettes containing guar gum, raises concern regarding addictiveness. The provided clinical tests show no effect of adding guar gum on inhalation facilitation and nicotine uptake, but these data are limited and do not address the previously identified concern regarding MAO inhibition. In the independent re-evaluation of the of mainstream smoke data reported in the industry report (see Section 4 of this Chapter and Annex II), almost all carbonyl concentrations increased with guar gum, and the increase in formaldehyde was evaluated as significant and relevant by the review panel. Other compounds that might be relevant for inhalation facilitation or nicotine delivery were not assessed in the industry report. Altogether, an influence on addictiveness or inhalation facilitation due to addition of guar gum to tobacco cannot be ruled out.

5.8.7 Characterizing flavor

Whether the additive guar gum can be responsible for introducing a characterizing flavor or odor to tobacco products has not been addressed in the industry report. Guar gum itself is flavor- and odorless and it is not likely that it leads to a characterizing flavor or odor in unburnt tobacco. However, combustion of sugars and carbohydrates in guar gum is known to contribute to complex aromas. Moreover, according to SCENIHR several other flavoring compounds were reported that are due to pyrolysis reactions of guar gum (2). It is not clear to what extent pyrolysis products of guar gum, such as hydroxymethylfurfural and furfural (4), can contribute to a characterizing flavor and/or odor of burned tobacco. However, even in the absence of a characterizing flavor, these flavoring compounds singly or in combination with other smoke constituents can contribute to the attractiveness of smoking by improving smoke flavor and masking its bitter taste. It is a notable shortcoming that

these effects of guar gum and its pyrolysis products were not assessed.

5.8.8 Overall conclusion on additive

The industry evaluated guar gum application levels of up to 1.5%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Guar gum is a non-volatile complex mixture consisting of compounds that undergo pyrolysis. Although no new pyrolysis experiments were performed, some of the listed pyrolysis products (furfural, Carc. 2; benzene, Carc. 1A, Muta 1B; toluene, Repr 2; 2-butenal, Muta 2) have CMR properties. The classified carcinogen furfural has been listed as pyrolysis product but was not included in the chemical comparative testing experiments. Also, application of guar gum caused an additive-level related increase of carbonyl compounds, especially for formaldehyde, and cadmium in the chemical analysis of mainstream smoke. The review panel concludes that it cannot be ruled out that use of guar gum as an additive contributes to an increase of CMR properties of mainstream smoke. Finally, inhalation toxicity was not evaluated in the industry report for the additive itself or its pyrolysis products. Thus, given the application level of up to 1.5% and based on the presented data, it can be concluded that use of guar gum as an additive in cigarettes and RYO tobacco is with concern regarding toxicity.

The influence on inhalation facilitation and addictiveness have not been assessed adequately. Aldehydes that are known MAO inhibitors were increased at tested levels raising concerns regarding addictiveness. Further, acetic pyrolysis products of guar gum might decrease the smoke pH, possibly making inhalation more palatable. Thus, concerns regarding addictiveness or inhalation facilitation due to addition of guar gum to tobacco cannot be ruled out. The impact of guar gum on tobacco flavor has not been addressed in the industry report. However, pyrolysis of guar gum is known to cause formation of flavoring compounds, which may improve smoke flavor and thereby increase the attractiveness of tobacco products.

The main concerns of the review panel with regards to using guar gum as an additive in cigarettes and roll your own tobacco are the possible enhancement of addictiveness due to increased emission of aldehydes that are known MAO inhibitors, the carcinogenicity of pyrolysis products (furfural, benzene, toluene, 2-butenal), an increase of toxic compounds (especially formaldehyde and cadmium) in the emissions, and the unaddressed flavoring properties that enhance attractiveness.

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5.9 Report of Licorice

5.9.1 Abstract

Licorice is a natural extract of the root of the licorice plant with the sweet tasting compound glycyrrhizin as the most relevant ingredient. Licorice is used in cigarettes in amounts up to 1.2%. In the industry report three levels are tested 0.6% (Low) 1.2% (Max) and 1.8% (Max-plus). Licorice is a complex mixture of mainly non-volatile compounds. The reported pyrolysis products furfural alcohol (11.7%, Carc. 2), phenol (1.4%, Muta. 2) and furfural (0.2%, Carc. 2) have CMR properties, and diacetyl (4.1%) is known to cause obstructive lung disease. The industry concluded in their report that application of licorice did not result in significant effects regarding smoke chemistry,

toxicity, addictiveness, inhalation facilitation or characterizing flavor. The review panel concluded that the industry reports provide data of low quality (see Chapter 4). The assessments of toxicity, addictiveness, inhalation facilitation, and characterizing flavor were considered to be insufficient. The main concerns of the review panel with regard to using licorice as an additive in cigarettes and roll your own tobacco are the CMR properties and toxicity of some pyrolysis products (especially furfuryl alcohol, phenol, furfural, and diacetyl), the carcinogenicity of constituents (cadmium), and its flavoring properties that enhance attractiveness.

5.9.2 Background

Licorice is a natural extract of the root of the licorice (*Glycyrrhiza glabra*) plant. This extract is an incompletely defined complex mixture of compounds. More than 400 compounds were isolated from *Glycyrrhiza* species. Previous assessments of scientific data on licorice as an additive in tobacco products have identified several concerns (1-3).

Regarding toxicity: Licorice extract contains polysaccharides that lead to comparable hazards as sugar additives (e.g. aldehydes formation). Chronic licorice uptake can lead to hypokalemia, hypernatremia, and water retention. Thus, it needs to be clarified whether exposure from tobacco can induce systemic toxicity.

Regarding addictiveness: Glycyrrhizin (also called glycyrrhizic acid) is a bronchodilator, so it may facilitate inhalation and nicotine uptake. It is not clear whether the levels present are sufficient for this effect, although a synergistic effect with other compounds in cigarette smoke (or other forms of exposure such as food) may be possible. The additive improves the organoleptic properties of tobacco smoke, making the harsh cigarette smoke palatable (thereby facilitating inhalation). Pyrolysis of licorice has been shown to lead to the formation of acetaldehyde and other aldehydes, which have the potential to increase the addictiveness of cigarette smoke via monoamine oxidase (MAO) inhibition (3).

Regarding characterizing flavor: The most relevant compound in licorice extract is the sweet tasting triterpene glycoside glycyrrhizin. Dried extracts of the roots of the licorice plant, which may contain between 4 % and 25 % glycyrrhizin, are used in products such as licorice candy, herbal teas, and alcoholic beverages. In addition, licorice extracts are used to improve the organoleptic properties of tobacco smoke. The taste and flavor of tobacco with added licorice and or licorice root are described as sweet, woody, and round.

5.9.3 Literature review

The industry report provides two literature overviews for licorice, one regarding the additive in general and a second one regarding licorice when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4). For example, some important sources were not used (i.e. ECHA). Relevant issues, such as physiological functions/properties or effects by inhalation are not sufficiently covered.

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.9.4 Chemistry and Pyrolysis products

Licorice and glycyrrhizin are widely present and/or used in foods, tobacco, and other herbal products. Licorice extract powder is used as tobacco additive to affect the aroma properties. The report provides information on the supplier, purity, lot specification and compliance with relevant EU-regulations for food. A constituent analysis was provided in the industry report, confirming a high proportion of carbohydrates (74.1%), while the content of sugars was 11.9 %. Further, the tested extract contained 9.1% glycyrrhizin.

Application levels: The licorice levels applied in test cigarettes varied between 0.6% and 1.8% (target levels Low 0.6%; Max 1.2%; Max-plus 1.8%; concentrations actually achieved were 0.42%, 1.05%, and 1.78%, respectively.

Transfer of licorice into mainstream smoke: The industry stated that components of licorice extract are unlikely to transfer intact into the mainstream smoke due to the complex chemical composition and non-volatile nature of the natural constituents. There were no published studies on the transfer of licorice extract into tobacco smoke presented. Transfer of glycyrrhizin was thought to be unlikely, since it has not been detected in pyrolysis experiments (4, 5). No new experiments have been performed to study transfer of licorice and its constituents, especially glycyrrhizin, into mainstream smoke.

Pyrolysis experiments: The industry report takes reference to the study by Baker and Bishop (6) stating that pyrolysis experiments are in good agreement with transfer/pyrolytic behavior of volatile additives that are added as small amounts. For non-volatile compounds, including licorice, pyrolytic experimental setups is not in a good agreement with MSS. According to Purkis *et al.* (4), licorice extract underwent full degradation when heated up to 900°C. Baker and Bishop had recorded pyrolysis patterns, finding acetic acid (42%), acetol (11.9%), furfuryl alcohol (11.7%), diacetyl (4.1%), acetol acetate (2%), phenol (1.4%), cresol (0.2%), pyridine or pyrrole (0.2%), and furfural (0.2%) (7). Carmines *et al.* show that pyrolysis of block licorice extract produces several compounds such as carbonyls, diacetyl, acetic acid, furfural, phenol, catechol, etc. (5).

Chemical analysis of mainstream smoke: For comparative testing of mainstream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.6 to 1.8 % licorice).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, is included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F) . In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

The literature review showed that the addition of licorice into tobacco products increased the emission of several compounds of toxicological concern in the mainstream smoke, such as carbonyls (mainly formaldehyde), catechol, NH3, several PAHs, phenol/cresols, and metals (Cd, As) (5, 6, 8, 9). In the comparative testing experiments NNK levels in the "Max" test cigarette (1.2% licorice extract) exceeded the variability of the 3R4F reference cigarette, but a similar increase was not present for the "Low" and "Max-plus" application levels. The absence of a dose-response and the fact that the standard deviation of the measurement is disproportionately high for the "Max" application level indicates that this effect might not be additive-related. On the other hand, the average level of cadmium was 34 % higher in "Max-plus" and 30 % higher in "Low" cigarettes, compared to the reference (no licorice supplement). These differences were just below the variability of the 3R4F monitor cigarette (3R4F Var99% = 34.5%). The corresponding increase at the "Max" application level was 18 %. The analysis performed by industry did not result in any overall statistically significant increase or decrease of any of the tested chemical compounds. The industry report concludes that an inclusion of 0.6 - 1.8% of licorice in tobacco does not affect the smoke chemistry significantly.

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). No increase of carbonyls was found at the tested levels.

Evaluation by the review panel: The submitted report on licorice examines the pyrolysis and the levels of harmful and potentially harmful constituents in cigarette smoke. However, the transfer rate and some aspects of smoke chemistry not were covered comprehensively. According to the industry report, licorice is applied up to 1.8% (Max-plus) in tobacco products. It is a complex mixture of compounds that mostly undergo pyrolysis. The industry concluded that no statistically significant and consistent additive-level related increases or decreases were recorded for any smoke constituent. An increase in cadmium was apparent that has not been acknowledged by the industry due to their statistical measures. Emission of some known pyrolysis products of licorice with CMR or inhalation toxic properties has not been addressed. Therefore, the review panel concludes that the use of licorice as an additive leads to the formation of toxic substances in the tobacco smoke, but the provided data are not sufficient to draw further conclusions.

5.9.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation. These are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – ASSESSSED

As discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relatively extensive evaluation of the toxicity of licorice when ingested. Glycyrrhizin is a naturally occurring triterpenoid saponin found in the extracts of roots and rhizomes from the licorice plant. Dried extracts of the roots of the licorice plant, which may contain between 4% and 25% glycyrrhizin, are present in licorice confectionery, licorice herbal teas, and in some health products. Moderate chronic or high acute exposure to glycyrrhizin and its metabolites has been demonstrated to cause several transient systemic alterations including increased potassium excretion, sodium and water retention, body weight gain, alkalosis, suppression of the renin-angiotensin-aldosterone system, and hypertension (10-12).

Several glycyrrhizin salts and various components of licorice containing glycyrrhizin have been investigated in a number of tests for mutagenicity and/or genotoxicity (10-12). Overall, although some positive findings were reported, the available data indicated that glycyrrhizin and its related salts are not genotoxic *in vitro* or in vivo. The available data indicated that glycyrrhizin and its salts do not have carcinogenic activity (10-12). Ammonium and disodium salts of glycyrrhizin at doses

of less than or equal to 1.5 g/kg bw per day have been evaluated in several developmental toxicity studies in mice, rats, hamsters, and rabbits (13, 14). In one of these studies, embryotoxicity was observed, but overall the data indicated that glycyrrhizin and its salts are not teratogenic (15).

The industry report refers to JECFA and the SCF conclusion that when ingested, licorice has no carcinogenic, mutagenic/genotoxic or reprotoxic properties, which is based on the evaluation of glycyrrhizin exposure in humans, based on oral exposure (10, 16). These data suggest that an intake of 100 mg/day glycyrrhizin would be unlikely to cause adverse effects in the majority of adults, although consumers with a high intake of licorice confectionery or herbal tea containing licorice may be exposed to glycyrrhizinic acid at more than 100 mg per day. Glycyrrhizin is not considered to be an irritant or a sensitizer, according to the CIR Expert Panel, at the current maximum concentration of use in the cosmetics industry. Licorice and its derivatives are widely used in many consumer goods, such as foods, flavorants, cosmetics, and medicines, so there is a long history of consumer exposure to this additive, although this is not always relevant for exposure by inhalation. In addition, it is not known to what extent the compositions of the extracts used are similar.

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

The inhalation toxicity of licorice is not evaluated in the industry report. Also, information regarding metabolite formation and their possible toxicity is lacking. Since licorice is composed of a mixture of non-volatile compounds, their transfer in its unburnt form to mainstream smoke is unlikely. However, as glycyrrhizin has been reported to act as a bronchodilator, transfer experiments should be performed to assess levels of glycyrrhizin in mainstream smoke.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The pyrolysis products identified in the report were not evaluated in terms of oral nor inhalation toxicity. Moreover, the identification of pyrolysis products was based on literature applying the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of licorice were not determined. Based on assessment by the review panel (see Annex III – "Pyrolysis product table") it was concluded that three of the reported pyrolysis products had CMR properties (furfuryl alcohol, Carc. 2; phenol, Muta. 2; furfural, Carc.2). Further, pyrolysis products with toxicologically relevance have been reported, especially diacetyl which is classified with specific target organ toxicity, causing severe obstructive lung diseases (see "Brief statement on known risks and missing data on diacetyl").

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

For comparative testing, the report includes both a literature review and new studies. The results of the *in vitro* studies did not reveal statistically significant and meaningful increases for the mutagenicity, cytotoxicity and genotoxicity for any of the samples (single additive or mixture; total particulate matter and gas vapor phase), when licorice extract powder was tested as a single additive up to 1.8%, or as part of a mixture. In support of their results, the industry report summarized results from several *in vivo* studies (e.g. (5, 17, 18)) with various forms of licorice extract applied to cigarette tobacco suggesting that adding licorice extract to cigarette tobacco at various levels (e.g. ≤5 % of tobacco) does not discernibly alter the smoke chemistry or biological effects usually associated with mainstream cigarette smoke.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 0.6-1.8% licorice does not

increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, the review panel concluded that the provided assessment is not sufficient as there were clear limitations in the approach and methodology. Toxic effects by ingestion are evaluated but are not relevant for the evaluation of the toxicity of licorice when used as a tobacco additive. The report does not evaluate the toxic, genotoxic and carcinogenic potential of the products formed by pyrolysis of licorice as such. In the evaluation by the review panel, three pyrolysis products were found to be classified with CMR properties (furfuryl alcohol, Carc. 2; phenol, Muta. 2; furfural, Carc. 2), while another pyrolysis product is known to cause severe obstructive lung disease (diacetyl). The levels of these pyrolysis products have not been assessed in the chemical analysis of mainstream smoke. Overall, a contribution to increased CMR properties upon application of licorice as an additive cannot be ruled out.

5.9.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: Pyrolysis of licorice has previously been shown to lead to the formation of acetaldehyde and other aldehydes, which have the potential to increase the addictiveness of cigarette smoke via monoamine oxidase (MAO) inhibition (3).

Inhalation facilitation: The major active principle of licorice is the sweet tasting triterpene glycoside glycyrrhizin. Glycyrrhizin is a bronchodilator and may thereby facilitate inhalation and nicotine delivery (1, 3). Further, decrease of smoke pH leads smokers to "increase their smoking frequency and inhale the smoke more deeply to enable a higher absorption of nicotine in the airways", as well as decreases the harshness and irritation of the smoke encouraging them "to develop a smoking habit" (7). The industry report indicates that pyrolysis of licorice leads to the formation of acids. Therefore, addition of licorice can potentially decrease smoke pH and could then reduce harshness of smoke and facilitate inhalation.

Industry reports: The presented data on bronchodilation is limited, but the studies consulted in the industry report (19, 20) do not support the hypothesis that licorice, as an additive to cigarettes or RYO tobacco, has a bronchodilating effect during smoking. However, according to one of these studies possible effects on addictiveness cannot be excluded as available data is scarce (19). Moreover, independent studies support a bronchodilating effect of glycyrrhizin in mice (1, 3, 21, 22).

As an indirect estimate of inhalation facilitation and nicotine uptake properties of licorice, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added licorice to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of licorice on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from licorice. Specifically, none of the reported studies did asses the capacity of metabolites and pyrolysis products of licorice on monoamine oxidase inhibition. There were also no studies reported assessing the effects of licorice on nicotine bio-availability and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores. Moreover, the "Max-plus" product, containing 1.8% of licorice, was not tested.

Evaluation by the review panel: The provided data show no effect of adding licorice on inhalation facilitation and nicotine uptake, but these data are limited and do not address the previously identified concern regarding MAO inhibition. In the independent re-evaluation of the of mainstream smoke data reported in the industry report (see Section 4 of this Chapter and Annex II), no consistent increase in carbonyl emissions at the tested levels was detected. Other compounds that might be relevant

for inhalation facilitation or nicotine delivery were not assessed in the industry report. Altogether, there is insufficient evidence to rule out an influence on addictiveness or inhalation facilitation upon application of licorice as an additive in cigarettes and RYO tobacco.

5.9.7 Characterizing flavor

Although licorice is a known flavoring agent, it is not possible to conclude whether it can be responsible for introducing a characterizing flavor or odor to tobacco products, on the basis of the evidence presented in the industry report. This is primarily due to the many uncertainties relating to how the evaluation was conducted and how the data might be interpreted.

The industry report concludes that based on the different sensory methodologies used (clustering, "In/Out" test and CATA testing), the addition of the single licorice extract powder at the Low 0.6 % and Max 1.2 % concentrations to test cigarettes did not result in a characterizing flavor. Specifically, the report indicates that overall 14 attributes were identified by three trained panelists, with "Licorice" not mentioned as an attribute of the two test cigarettes containing licorice. The Test products were rather described as "dried fruits" and "sweetish". As described in the report, cluster analysis using 15 consumers screened for their sensory ability: The two test products containing different levels of licorice were allocated to different clusters. Licorice Low (0.6%) was allocated to cluster 6 (most frequently mentioned attribute is "dried tobacco leaves" with a score of 78, cluster scores somewhat higher for "dried fruits" with a score of 46 and for "rum" with a score of 21 than other clusters). Licorice Max (1.2%) was allocated to cluster 12 (most frequently mentioned attribute "dried tobacco leaves" with a score of 90, cluster scoring somewhat higher for "dried tobacco leaves" and for "licorice" (score of 28) than other clusters). Both licorice test products were rated with an "Out" score of 0 in the "In/Out" test and were therefore not further assessed in the CATA test.

There are fundamental methodological flaws with using the "In/Out" test of two products with licorice concentrations to make the conclusive statement that licorice additive does not result in a characterizing flavor. Additionally, other vital information is missing from the industry report that could be a determining factor in whether or not licorice would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which licorice was incorporated in the tobacco product, as well the quantity of the remaining licorice. Furthermore, the methods used to select sensory assessors are not considered valid. The reported study used assessors (adult smokers) who were likely to have lower sensitivity to the odor of licorice in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits (i.e. young, non-smokers). In addition, the screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor, and because the selection criteria identified in advance of testing were often not applied.

Evaluation by the review panel: Even in the case of absence of characterizing flavors, the addition of licorice can increase the attractiveness of cigarettes and RYO by modifying the perceived flavor, taste or odor of the final product. Industry report does not contain any information about the potential increase of attractiveness on cigarettes and RYO by the addition of licorice. The impact of licorice on tobacco flavor is likely to have been underestimated in the reported study.

5.9.8 Overall conclusion on additive

The industry evaluated licorice application levels of up to 1.8 %, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry's assessment of toxicity,

addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Licorice is applied in tobacco up to 1.8 %, and is a non-volatile complex mixture consisting of compounds that undergo pyrolysis. No new pyrolysis experiments were performed, however some of the listed pyrolysis products had CMR properties (furfuryl alcohol, Carc. 2; phenol, Muta. 2; furfural, Carc. 2) or specific target organ toxic properties (diacetyl). In addition, diacetyl is believed to contribute to generation of chronic obstructive pulmonary disease in smokers (see "Brief statement on known risks and missing data on diacetyl"). Contribution to toxicity and CMR properties has not been ruled out and is of concern. The absence of new experiments to assess levels of the bronchodilator glycyrrhizin in the mainstream smoke does not allow ruling out of a possible effect on inhalation facilitation. Although some pyrolysis products of licorice are known precursors for MAO inhibitors, no increase was found for analyzed aldehydes at tested levels. The impact of licorice on tobacco flavor is likely to have been underestimated in the reported study and, as licorice is used to enhance tobacco taste, there is still a major concern that it will increase the attractiveness of tobacco products, by attenuating bitterness and harshness of tobacco.

The main concerns of the review panel with regards to using licorice as an additive in cigarettes and roll your own tobacco are the CMR properties and toxicity of some pyrolysis products (especially furfuryl alcohol, phenol, furfural, and diacetyl), the carcinogenicity of constituents (cadmium), and its flavoring properties that enhance attractiveness.

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5.10 Report of Maltol

5.10.1 Abstract

Maltol naturally occurs in food, including bread, butter, beer, coffee and soy products. It is also used as a flavor in tobacco products in application levels up to 0.01%. In the industry report three levels are tested, 0.005% (Low), 0.01% (Max) and 0.015% (Max-plus). Maltol is not very volatile in ambient conditions and the transfer into mainstream smoke is mainly intact (transfer rate 4.3 to 5.2%). The industry concluded in their report that application of maltol did not result in significant effects regarding

smoke chemistry, toxicity, addictiveness, inhalation facilitation. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness and inhalation facilitation. Moreover, (characterizing) flavoring properties were not assessed. Considering the low application level, transfer rate and pyrolysis rate, the review panel concluded that an influence on smoke chemistry of the compounds analyzed here and toxicity of maltol when used as an additive in cigarettes and RYO tobacco is unlikely, but cannot be ruled out. The main concern of the review panel with regards to using maltol as an additive in cigarettes and roll your own tobacco is its flavoring properties that enhance attractiveness.

5.10.2 Background

Maltol is used as a flavor in tobacco products, but also occurred naturally in food, including bread, butter, beer, coffee and soy products. During baking and roasting, simple sugars are partly converted to maltol. Maltol is used as a synthetic compound (CAS 118-71-8) in tobacco products. Previous assessments of scientific data on maltol as an additive in tobacco products have identified several concerns (1, 2):

Regarding toxicity: Concerns regarding possible genotoxicity of maltol could not be excluded so far. Inhibition of GABAA receptor response has been reported in presence of maltol.

Regarding Addictiveness: There are currently no data that suggest an addictive effect of Maltol.

Regarding characterizing flavor: Maltol and derivates are used as taste enhancers in consumables and is used as flavor in tobacco products.

5.10.3 Literature review

The industry report provides two literature overviews for maltol, one regarding the additive in general and a second one regarding maltol when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4). For example, some important sources were not used (i.e. ECHA). Relevant issues, such as physiological functions/properties or effects by inhalation are not sufficiently covered.

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.10.4 Chemistry and Pyrolysis products

The report provides a description of maltol as additive, including specification for the substance used to manufacture the cigarettes that were analysed in the comparative testing program. Information is provided on the supplier, purity, lot specification and compliance with relevant EU-regulation. The report also states that the referenced lot represents the typical characteristics of the additive maltol.

Application levels: Maltol levels applied in test cigarettes varied between 0.005% and 0.015 % (target concentrations Low 0.005 %; Max, 0.01 %; Max-plus 0.015 %; with achieved levels 0.0045%, 0.0079%, and 0.0111%, respectively).

Transfer of maltol into mainstream smoke: According to the industry, no studies have yet been published on the transfer of maltol into the mainstream smoke (MSS). In the industry report, transfer

rates between 4.3 % and 5.2% were determined for test cigarettes that contain 0.005% (Low), 0.01% (Max) or 0.015% (Max-plus) maltol.

Pyrolysis experiments: Previous studies confirmed that maltol does not undergo thermal degradation under pyrolysis conditions and transfers intact into cigarette smoke. Baker and Bishop identified acetoxymethyl pyranone (0.2%) as one minor pyrolysis product (3). No new experimental data or internal information has been submitted in the industry report.

Chemical analysis of mainstream smoke: For comparative testing of mainstream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.005 – 0.015 %).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, is included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

In the comparative chemical analysis of main stream smoke, the variability of nitrogen oxides (NO and NOx) and water exceeded the variability of the 3R4F monitor cigarettes. The same was observed for carbonylic compounds. However these increased levels did not correlate with the application levels of maltol. Moderately increased levels of carbonylic compounds were found in all “Low” and in some “Max” samples, while no “Max-plus” cigarette was affected. The industry concluded that no statistically significant and consistent additive-level related increases or decreases were recorded for any smoke constituent.

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). As the data was of poor quality, it was difficult to draw conclusions on the influence of maltol. Although there were increased levels of carbonyl formation, the review panel concluded that these may not be attributable to maltol, as the compound levels did not increase with increasing levels of maltol in the test cigarette.

Evaluation by the review panel: The submitted report on maltol examines the transfer, pyrolysis, as well as the levels of harmful and potentially harmful constituents in cigarette smoke. However, there are several limitations in the comparative testing approach. Maltol is applied in very low levels (0.015 %), with a low transfer rate into mainstream smoke (4-5 %). In addition, pyrolysis experiments showed that most of the compound stays intact. The industry concluded that no statistically significant and consistent additive-level related increases or decreases were recorded for any smoke constituent. Although, there were limitations in the approach and methodology, the members of the review panel

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

conclude that increased formation of toxic substances in the tobacco smoke due to application of maltol at levels < 0.015% is unlikely, but cannot be ruled out.

5.10.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation, these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – PARTLY ASSESSSED

As discussed in Chapter 4, evaluation of the oral toxicity of a compound has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relative extensive evaluation of the toxicity of maltol when ingested. An ethyl maltol study was provided to rule out any carcinogenic effects of maltol. Only one reproductive and developmental study was mentioned but cannot be used because of the appearance of a viral infection in all rats during tests (4).The industry report is almost exclusively based on conclusions by the EFSA panel and JECFA committee (5-7). In relation to these, they consider maltol to be non-genotoxic, non-mutagenic. However, multiple studies cited in the report have shown variations in organ weights in animals exposed to the highest doses but are not mentioned in the conclusion. Moreover, in their report, the SCENIHR panel indicated that maltol has an effect on CNS stimulation by inhibiting the response of GABAA receptors (1, 8). This issue is not addressed in the industry report.

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

The inhalation toxicity of maltol is not evaluated in the industry report. Literature on maltol toxicity by inhalation seems very limited to publication from tobacco industry and there are no studies available in the ECHA registration dossier. Ethyl-maltol seems to be much more studied, particularly because of its frequent use in e-cigarettes. It is unfortunate that the industry did not mention these studies, in the same way as they did in the evaluation of the additive by ingestion.

Due to the absence of provided data, experts from the review panel have used the TTC concept for a provisional assessment: Using the TTC concept (Threshold of Toxicological concern), initial risk assessments indicate that the daily exposure would be slightly above the TTC of Cramer class III compounds (90 µg/day), but well below the TTCs for Cramer class II and I. EFSA has classified maltol as a Cramer class II compound, although reclassification into Cramer class III was previously proposed (9). Recently, the Joint FAO/WHO Expert Committee has concluded that a dietary exposure of 1 mg maltol per kg bw does not raise safety concerns; however, the previous ADI was withdrawn because of data gaps (10). Caution is necessary, if this conclusion was extended to inhalation. However, the expected exposure via inhalation of cigarette smoke is approximately 5000-fold lower than the proposed safe level (based on 1 mg/kg bw) for oral exposure. Consequently, it could be assumed that significant toxic effects of maltol as tobacco additive are unlikely.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The industry states in its report that maltol is practically not pyrolyzed under the conditions of the experiment. It is transferred intact to 99.8% in smoke, and to 0.2% as acetoxymethyl pyranone. This pyrolysis product was not evaluated by the industry in terms of oral nor inhalation toxicity. Based on assessment by the review panel it was concluded that acetoxymethyl pyranone is currently not classified as a CMR carcinogen (see Annex III – Pyrolysis product table).

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

For comparative testing, the report includes both a literature review and new studies. Based on the performed comparative *in vitro* experiments, that did not identify significant effects, the industry report concludes that at inclusion of 0.005 – 0.015% of maltol in tobacco does not increase the CMR properties of the mainstream smoke.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 0.005 – 0.015 % of maltol in tobacco does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, there were several methodological limitations in the comparative testing approach, and no data was presented on the inhalation toxicity of maltol and its pyrolysis product. In spite of these limitations in the approach and methodology, the members of the review panel conclude that contribution to toxicity or CMR properties upon application of maltol as an additive in cigarettes and RYO tobacco is unlikely, given its low application level, transfer rate and pyrolysis rate, but cannot be ruled out.

5.10.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: No concerns were identified regarding possible effects of maltol or its pyrolysis products on inhalation facilitation.

Inhalation facilitation: No concerns were identified regarding possible effects of maltol or its pyrolysis products on inhalation facilitation.

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of maltol, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added maltol to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of maltol on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. There were also no studies reported assessing the effects of maltol on nicotine bio-availability and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores.*

Evaluation by the review panel: The provided data show no effect of adding maltol on inhalation facilitation and nicotine uptake, but these data are limited.. Altogether, an influence on addictiveness upon application of maltol as an additive in cigarettes and RYO tobacco is unlikely but cannot be ruled out.

5.10.7 Characterizing flavor

Whether the additive maltol can be responsible for introducing a characterizing flavor or odor to tobacco products has not been addressed in the industry report. However, as maltol is used as taste enhancer and flavoring, such an effect is seen as very likely and the fact that a characterizing flavor assessment was not conducted by the industry is a notable omission. Even in the case of absence of characterizing flavor, the addition of maltol could alter the MSS flavor, taste or odor leading to more attractive products.

5.10.8 Overall conclusion on additive

The industry evaluated maltol application levels of up to 0.015%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, and nicotine uptake. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient or even completely missing. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Maltol is applied in low levels in tobacco (up to 0.015 %), and is a non-volatile compound with low transfer rate (4-5 %) that mostly stays intact during pyrolysis (>99 %). No new pyrolysis experiments were performed and toxicity of pyrolysis products was not assessed. Also, inhalation toxicity is not evaluated in the industry report. Nevertheless, the review panel concluded that the low application level, transfer rate and pyrolysis rate suggest that an influence of maltol, when used as an additive in cigarettes and RYO tobacco, on smoke chemistry and toxicity is unlikely, but cannot be ruled out. Influence on addictiveness and inhalation facilitation is unlikely, but cannot be ruled out. In addition, maltol is known to be a flavoring additive but no data regarding characterizing flavor assessment have been provided. The addition of flavorings make tobacco products attractive and palatable, and is therefore cause for concern.

The main concern of the review panel with regards to using maltol as an additive in cigarettes and roll your own tobacco is its unassessed flavoring properties that enhance attractiveness.

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5.11 Report of Menthol

5.11.1 Abstract

Menthol is used as flavor in food products and is used as a tobacco additive in amounts of 0.0002 to 10% in test cigarettes. In the industry report three levels are tested 0.1% (Low) 1.2% (Max) and 1.8% (Max-plus). The characteristic peppermint-like taste, sensation of freshness and pleasant coolness of declining heat is noticeable when menthol is added between 0.6% and 1.2% of tobacco weight. Furthermore, menthol can mask the harshness and irritating properties of smoke and nicotine and facilitate inhalation at substantially lower levels (0.03%). Menthol is also discussed to increase the addictiveness of adolescent smokers. Menthol is a volatile compound. The industry reports transfer rates for menthol into mainstream smoke of 9.1 – 11.1%, while rates up to 30%have been described in earlier reports. During pyrolysis, menthol transfers mostly intact (99%) and pyrolysis products have no CMR properties. The industry concluded in their report that application of menthol did not result in significant effects regarding addictiveness or inhalation facilitation. Concerning smoke chemistry, no overall significant increase of toxicants was acknowledged by industry. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. Importantly, menthol's ability to facilitate inhalation via activation of the cooling receptor TRPM8 was not addressed. The main concern of the review panel with regard to using menthol as an additive in cigarettes and roll your own tobacco is its physiological effect (TRPM8 activation) at even low levels that do not lead to a characterizing flavor. This physiological effect leads to inhalation facilitation and promotes inhalation of tobacco smoke, which is of high relevance for new and experimenting smokers. Consequently, this property contributes to increased addictiveness of nicotine and indirect increases in toxicity.

5.11.2 Background

Menthol is a natural compound found in several plants of the mint family e.g. the peppermint, cornmint, and spearmint herbs. When consumed it imparts a minty taste and smell, and has a characteristic cooling effect. Menthol is also produced synthetically for commercial use, and is widely used in the food, flavor, oral hygiene, cosmetic, and pharmaceutical industries. It is also one of the most commonly used additives in cigarettes, roll-your-own (RYO) and smokeless tobacco products. It is a monocyclic terpene alcohol that is used primarily for its chemosensory effects of creating perceptions of a cooling minty taste and smell. In contrast to other priority additives, there are comprehensive independent studies available, such as the report by the US Food and Drug Administration (FDA) Tobacco Product Scientific Advisory Committee (TPSAC). Previous assessments of scientific data on menthol as an additive in tobacco products have identified several concerns (1, 2).

Regarding Addictiveness: Menthol is thought to increase the addictiveness of cigarettes in various ways, including altering of nicotine levels and function, and altering bioavailability of nicotine (3). In addition, menthol activates the cooling receptor TRPM8 and triggers sensations of "pleasant" cooling and of improved breathing. Due to this cooling effect, menthol can mask the irritating and harsh properties of cigarette smoke and, consequently, increase the tobacco attractiveness. Enhanced inhalation of irritating fumes was confirmed in animal experiments, depending on TRPM8 activation. Consistently, a preferential use of mentholated cigarettes was demonstrated during initiation in the United States. The TPSAC report further summarized that menthol is likely to increase addictiveness of adolescent smokers. In the context of smoking, the properties to ease inhalation and to suppress

physiological responses towards respiratory irritation are strongly associated. However, the capacity to facilitate inhalation of smoke might differently affect experimenting/initiating and established smokers. Consequently, risk assessments of menthol should comprehensively consider both intrinsic properties and physiological responses for different populations of smokers.

Regarding toxicity: Menthol suppresses respiratory irritation by smoke and smoke constituents (e.g. acrolein, cyclohexane). Facilitation of inhalation due to menthol increases the dosage of nicotine but also of other smoke toxicants.

Regarding characterizing flavor: Menthol has mint-like aroma properties and is used at levels ranging from imperceptible to imparting a characterizing flavor.

5.11.3 Literature review

The industry report provides two literature overviews for menthol, one regarding additive in general and a second one regarding menthol when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4).

The industry report states that there is no evidence in the literature to suggest an addictive effect of menthol. However, many independent studies suggest an enhancement of tobacco and nicotine dependence by menthol. For example, Henderson *et al.* (4) concluded that: "In a conditioned place preference (CPP) assay, we observed that menthol plus nicotine produces greater reward-related behavior than nicotine alone". Additional literature from independent research is detailed in section 8 of this chapter.

Other findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.11.4 Chemistry and Pyrolysis products

The report provides a description of menthol as additive, including specifications for (-)-menthol (CAS 2216-51-5) that was used to manufacture the cigarettes that were analyzed in the comparative testing program. Submitted data cover information on the manufacturer, purity and compliance with product characteristics set by US FEMA and FDA GRAS. The report states that the referenced lot represents the typical characteristics of the additive menthol, as used in cigarette manufacturing. Other applications of menthol (d-menthol, dl-menthol, peppermint oil, spearmint extracts/oils) are also listed. The report does not address different properties of menthol enantiomers.

Application levels: The menthol levels applied in test cigarettes varied between 0.6 and 1.2% (target levels Low 0.6 %, Max 1.2 %, Max-plus 1.8 %; actually achieved concentrations 0.552%, 1.142%, and 1.730%, respectively)

Transfer of menthol into mainstream smoke: Transfer rates for menthol into mainstream smoke (MSS) were found between 9.1 – 11.1%, in the submitted study performed by the industry using the ISO method. However, rates of about 30% have been described in earlier reports (5, 6). Industry stated that cigarette design features like filter ventilation and constructions to reduce tar (7), may have led to an underestimation of the transfer rates in their study, as compared to the unventilated cigarettes that were used in the earlier investigations (5, 6).

Pyrolysis experiments: No novel experimental studies have been performed to address pyrolysis

of menthol. The industry report refers to published studies by Baker and Bishop (8), showing that 99% menthol was remained intact, with some degradation to 0.9% menthone and 0.1% menthene. Furthermore, in pyrolysis experiments, Purkis *et al.* (6) demonstrated a 97.4% transfer of intact menthol into the gas phase, besides one non-identified degradation product. Besides the before mentioned studied pyrolysis of the additives by itself, Purkis *et al.* (6) have investigated the fate of menthol in burnt cigarettes in a smoking machine experiment. Summing up the menthol concentrations in mainstream and sidestream smoke and in butt and ash, they found a recovery of 83%. The apparent loss of 17%, indicating some combustion under these conditions, has not been reflected in the previous pyrolysis studies. However, based on the publications on single additive pyrolysis experiments, the industry concluded that menthol will hardly pyrolyze in a burnt cigarette. So the last reference was not taken into consideration and no new experimental studies have been performed.

Chemical analysis of mainstream smoke: For comparative testing of mainstream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.6 - 1.8 %).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, are described in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

Some constituents show variations in their concentrations in mainstream smoke after addition of menthol in relation to the additive free reference product. Levels of one tobacco specific nitrosamine (TSNA), N-nitrosoanatabine (NAT), were increased by 22.9% in the Max-plus product that contains 1.8% menthol. However, the industry did not acknowledge this effect as overall significant according to their applied statistical measures.

In addition to this assessment, studies that address the smoke chemistry of menthol cigarettes have been cited in the industry report. Based on these studies, the industry concluded that menthol does not substantially affect the composition of mainstream smoke (MSS) in general. However, in several studies, statistically significant increases of several smoke components were reported for cigarettes containing menthol compared to menthol-free controls (formaldehyde and 2-furfural (9), TPM, formaldehyde, resorcinol and lead (10), hydrogen cyanide, several carbonyls, HCs and PAH (11), etc.) These findings were either not confirmed or not assessed (i.e. 2-furfural, which is classified as a CMR carcinogen cat. 2 under the EC Regulation No 1272/2008) in the chemical analysis of mainstream smoke in the industry report. Overall, substantial variations of individual compounds have been described for comparative testing of machine generated smoke in previous

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

studies. These observed variations are not sufficiently discussed in the industry reports.

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for aldehydes presented by the industry (see Section 3.4 and Annex II). No increase of carbonyls was found at the tested levels.

Evaluation by the review panel: The submitted report on menthol examines the transfer rate, pyrolysis, and the levels of harmful and potentially harmful constituents in cigarette smoke. However, no new pyrolysis experiments were performed and there are limitations in the comparative testing approach. Increase of furfural (Carc. 2) that has been demonstrated in previous studies has not been followed up by industry. An apparent additive-level related increase in N-nitrosoanatabine (NAT) has not been acknowledged by industry as overall significant. Thus, the limitations in the approach and methodology do not allow the members of the review panel to draw conclusions regarding an influence on smoke chemistry due to application of menthol as an additive in cigarettes and RYO tobacco.

5.11.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – ASSESSSED

As discussed in Chapter 4, evaluation of the oral toxicity of an additive has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, in the report, the acute and chronic oral toxicity of menthol is evaluated, and the report concludes that menthol showed no evidence of genotoxic or carcinogenic properties based on *in vivo* studies in their literature review. Further, the industry report takes reference to the Classification, Labelling and Packaging (CLP) regulation (EC) No. 1272/2008 stating that menthol is not classified as CMR-compound. No studies regarding reproductive toxicity of menthol were identified, but since no European harmonized classification exists, it was concluded that menthol is not toxic to reproduction. Although data are relatively complete and include carcinogenicity, reproductive toxicity, and developmental toxicity, the conclusions have limited relevance for the evaluation of the use of menthol as a tobacco additive given the route of exposure studied. In addition, no information is provided on the exposure in smokers in relation to the general population, taking additional routes of exposure into account.

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

Only one inhalation study is mentioned in the report, and because of the lack of reliability of the measurement of the exposures, manufacturers stated that it did not allow for a conclusion to be drawn. At least four studies were excluded from the industry literature review for the following reason: “Additive not contained in cigarettes or RYO tobacco” despite the fact that they all relate to menthol. Three of them conclude that menthol can decrease respiratory sensitivity to irritants in cigarette smoke and thus facilitates the penetration of certain cytotoxic agents such as acrolein (12-14). Several comparative studies on health risks have been included in the report, concluding that there are no significant differences between subjects who smoked menthol cigarettes vs. those who have smoked non-mentholated cigarettes in respect to health risks. However, in some listed studies, increased incidences of stroke (15-17) and chronic obstructive pulmonary disease (18) were observed for some defined risk groups in relation to smoking menthol cigarettes. None of these studies interpreted in the report and their conclusions are not included or discussed. There is ongoing debate whether menthol increases the toxicity of cigarette smoke directly. However, menthol is expected to affect toxicant exposures indirectly due to increased puff volumes. This has been observed in some experimental settings (19, 20) but was not discussed by the industry. In addition, menthol was shown to increase the permeability of respiratory epithelia for chemicals,

toxicants or drugs (21).

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

Based on the literature and on the submitted data on transfer, it was concluded that menthol hardly pyrolyses. Consequently, is was stated that pyrolysis products are unlikely to affect the toxicity of cigarette smoke. However, the reference of Purkis *et al.* (6) that the transfer rate of menthol is only 83%, is not taken into consideration and also no assessments have been provided for menthone and menthene that had been identified as minor products. Further, no new experimental studies have been performed. Based on assessment by the review panel it was concluded that none of the reported pyrolysis products had CMR properties. One pyrolysis product (menthone, 0.9%) and menthol itself might be a skin irritant.

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

The toxic properties of MSS were determined, using extracts of the total particulate matter (TPM) or the collected gas-vapor phase (GVP). Based on the new experimental studies performed by the industry, including Ames Assays, Neutral Red Uptake Assays and *in vitro* Micronucleus Assays, the report concludes "that there were no statistically significant and meaningful increases for the mutagenicity, cytotoxicity and genotoxicity for any of the samples when menthol was tested."

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 0.6-1.8% menthol does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, the review panel concludes that the presented toxicological assessment of menthol is insufficient. There were several methodological limitations in the comparative testing approach. Furthermore, only CMR properties were considered in the *in vitro* testing approach and important studies on the inhalation toxicity of menthol and its pyrolysis products were omitted. The increase of furfural (Carc. 2) levels in mainstream smoke that has been reported in previous studies was not followed up in the industry report. Most importantly, indirect effects of menthol on toxicity (e.g. due to increased puff volume and increased pulmonary epithelium permeability to cigarette smoke toxicants) have not been addressed. Therefore, a direct and especially an indirect effect, of menthol as an additive in cigarettes and RYO tobacco, on the toxicity of mainstream smoke cannot be ruled out.

5.11.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: Menthol is thought to increase the addictiveness of cigarettes in many ways, among which altering of nicotine levels and function, masking of aversive sensory experiences, serving as a conditioned cue, and altering bioavailability of nicotine (3).

Inhalation facilitation: Menthol activates the cooling receptor TRPM8, which triggers (pleasant) sensations of cooling and enhances inhalation of irritating fumes (1).

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of menthol, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical*

test to compare the test cigarette with added menthol to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of menthol on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from menthol. There were also no studies reported assessing the effects of menthol on nicotine bio-availability and clinical markers of addiction, such as craving, withdrawal symptoms or dependence scores. Finally, none of the biological mechanisms by which menthol is thought to influence the addictive potential of nicotine were assessed (see below).

Evaluation by the review panel: The industry concludes that there was no effect of adding menthol at tested levels on inhalation facilitation and nicotine uptake. In the review panels opinion, these data are limited and do not address the previously identified concern of TRPM8 activation. The issue whether menthol can increase the addictiveness of nicotine was neither comprehensively, nor appropriately covered by the industry report. Industry did provide a pharmacokinetics study aimed to measure nicotine uptake by established smokers. In this study, no effects of menthol on nicotine-uptake were found. In contrast to substantial independent data, the industry's clinical study on facilitation of inhalation and nicotine uptake has little, if any, relevance to address the crucial role of menthol to promote inhalation during initiation. Notably, the menthol content (1.2%) was comparatively high, much higher than levels that are usually preferred by adolescents and young people (22). The submitted study only allows limited conclusions for established and adapted smokers, but does not cover the entire risk profile of menthol in regard to inhalation.

The statement on p. 169 that by the current state of scientific knowledge, it was concluded that the clinical study gave no circumstantial indications of increased addictiveness, is somehow misleading, as this study was not designed to determine addictiveness. In fact, industry claims that no validated or even suitable methods exist to demonstrate a significantly or measurably increased addictiveness by any tobacco additive at the stage of consumption (industry report, p. 168, last section). Further, they do not expect that such methods will be developed within the next years (p. 168 last section). Following these arguments, it would be impossible to determine for any additive, whether it affects addictiveness to a relevant degree as required by Article 7. However, the review panel does not agree with this reasoning, as there is ample relevant evidence for addictive effects of tobacco additives from independent sources. Further information regarding influence on addictiveness of menthol is provided below, see Section 7.

Importantly, the submitted report fails to recognize that properties of priority-listed additives to facilitate inhalation or nicotine uptake are not required to be significant or measurable at the stage of consumption according to Article 7. Consequently, it is not acceptable to disregard non-industry studies on model aerosols as relevant evidence for menthol-related effects on inhalation, because the additive was not applied in tobacco smoke (see report by Kleijnen Systematic Reviews: reason for exclusion of studies, ref. Willis et al (13)).

In conclusion, there is ample evidence from independent sources that shows that menthol affects the addictive potential of nicotine and facilitates inhalation. These effects of menthol were not properly addressed in the industry report and raise strong concerns whether application of menthol is compliant with Article 7 of the TPD.

5.11.7 Characterizing flavor

According to the report, menthol is applied at levels between 0.0002 and 10% in test cigarettes. However, the range in commercial cigarettes is hardly discussed. Importantly, it is claimed that only a menthol content of 1.2 % (but not 0.6%) leads to a characterizing flavor. In contrast, independent authors found that brands which contain menthol in between 0.1% and 1% of their tobacco weight impart a noticeable cooling sensation and mint-like flavor when inhaled (22). In fact, application

levels of menthol are as low as 0.03% (22). This raises question on the intended use of menthol levels that are far below the reported threshold to develop a characterizing mint-like flavor when inhaled. It is also not mentioned that the cooling effect that is crucial to alleviate irritations occurs at these comparatively low levels (23). No information is provided, about whether menthol is used as component in complex alternate flavors to modify the smell or taste tobacco. Further, additional content of peppermint/spearmint oil or extracts that could affect taste, sensation or other relevant product properties are not discussed in the industry report.

Although menthol is a known flavoring agent, it is not possible to conclude whether the additive menthol can be responsible for introducing a characterizing flavor or odor to tobacco products, on the basis of the evidence presented in the industry report. This is primarily due to the many uncertainties relating to how the evaluation was conducted and how the data might be interpreted. The industry report concludes that based on the different sensory methodologies used (clustering, "In/Out" test and CATA testing), the addition of menthol at levels from 0.6% (menthol Max) to test cigarettes did not result in a characterizing flavor, but there is a characterizing flavor in the menthol Max product (1.2%).

The menthol Min and Max products was rated as "Out" from the 40 consumers. The assessment of 72 products (reference products, positive controls and test cigarettes with priority additives) identified 12 distinct clusters. Cluster analysis using 3 trained panelists identified 14 attributes. The tests using 15 consumers allocated the two menthol products to cluster 1 with a score of 92. The menthol test products were rated with an out score of 2 (menthol Low) to 7 (menthol Max) out of 10. In the CATA test, the menthol Max test product was rated significantly higher for the attribute "menthol/peppermint" than the rest of the products, thus it has a characterizing flavor.

There are fundamental methodological flaws with using the "In/Out" test of two products with menthol concentrations to make the conclusive statement that menthol additive does not result in a characterizing flavor. Additionally, other vital information is missing from the industry report that could be a determining factor in whether or not menthol would impart a characterizing flavor. Such factors include the type of source material, the age of the material, the conditions under which it has been stored and storage time, the way in which menthol was incorporated in the tobacco product, as well the quantity of the remaining menthol. Furthermore, the methods used to select sensory assessors are not considered valid. The reported study used assessors (adult smokers) who were likely to have lower sensitivity to the odor of menthol in tobacco products than either the population at large, or the specific cohort at risk on account of their age and smoking habits (i.e. young, non-smokers). In addition, the screening methods used were of limited value, being mostly focused on evaluation of taste, rather than on odor, and because the selection criteria identified in advance of testing were often not applied. Moreover, the industry did not report any efforts to determine threshold of characterizing flavor, which can be everywhere between 0.6 and 1.2%.

Evaluation by the review panel: As a result of the methodological shortcomings, the impact of menthol on tobacco flavor is likely to have been underestimated in the reported study. According to the industry, only application levels of 1.2% and higher lead to a characterizing flavor, despite other evidence from previous studies. Importantly, even in the absence of characterizing flavors, the addition of menthol does increase the palatability and attractiveness of cigarettes and RYO by creating a cooling sensation even at low application levels.

5.11.8 Additional information based on independent data sources – as retrieved by WP 9 participants

Further information and discussion Regarding Addictiveness: Although the industry report concluded that menthol does not facilitate inhalation or nicotine uptake, several studies indicate the opposite. Ha *et al.* (24) showed an increase of cotinine in plasma in mice exposed to cigarette smoke containing l-menthol vs. mice exposed to reference cigarette smoke. Dunér-Engström *et al.*

(25) showed that menthol might increase the dissolution of nicotine in mouth via stimulation of salivary flow. Further, Shojaei *et al.* (26) did establish that nicotine could also influence transbuccal absorption. Moreover, a publication of Squier *et al.* (12) concluded that menthol increases the nicotine uptake. The study by Squier *et al.* (12) as well as other well-performed investigations, has been excluded from further considerations in the industry report for the reason that "the additive was not contained in cigarettes or RYO tobacco".

Menthol is used as an antitussive compound in pharmacology (for review see Dicpinigaitis et al.(27) and Eccles *et al.* (28)) and was shown to suppress strong irritancy induced by pro-tussive compounds in mice, dependent on TRPM8 activation (13). Similar effects had been found in guinea pigs earlier (29). Menthol was further shown to reduce cough sensitivity to inhaled capsaicin and to improve inspiratory flow in chronic cough patients (30). Data might suggest that menthol hardly affects toxicant exposure and health risks of experienced smokers who are adjusted to inhale hazardous smoke (31). However, during initiation and adaption of new smokers, supplemented menthol inhibits physiological warning and rejection responses, thus facilitating an easier and continued inhalation despite the harshness of smoke and irritating qualities of nicotine (32). This is consistent with the preferential use of mentholated cigarettes by adolescents and young adults (33). Taken together, menthol and other cooling agents affect the risks to adopt a regular smoking (i.e. inhalation) behavior, but have far less impact on health risks that are generally associated with cigarette smoking. Although the industry report refers to the pharmacological properties of menthol, some key-publications are not adequately discussed as for example Willis *et al.* (13); McKemy *et al.* (34); Yerger *et al.* (32); Ha *et al.* (24) and others that refer to its intrinsic property to enhance inhalation. This effect is also linked to an improved sensation of breathing and air-flow, especially under conditions of respiratory diseases or toxicant exposure. Some conclusions in the industry report are misleading. For example the statement "currently there is no published data in the literature which has investigated the relationship between the cooling sensation properties and facilitation of deeper inhalation" (p. 27) implies deeper inhalation as crucial criterion for facilitated inhalation. However, re-normalization of breathing patterns that are delayed or inhibited by toxicants is much more relevant, and this effect was confirmed for menthol and other TRPM8 agonists

Many independent studies also suggest an enhancement of tobacco and nicotine dependence by menthol. For example, Henderson *et al.* (4) concluded that: "In a conditioned place preference (CPP) assay, we observed that menthol plus nicotine produces greater reward-related behavior than nicotine alone". Wang *et al.* (35) summarized that: "menthol, likely by inducing a cooling sensation, becomes a potent conditioned reinforcer when it is contingently delivered with nicotine". Importantly, enhanced self-administration of nicotine in response to menthol was linked to TRPM8 activation, as this effect was also induced by an agonist lacking the typical peppermint-like taste. The study by Wang points to an interplay between TRPM8 activation and nicotine dependence, although further work is required to clarify the mechanisms. Furthermore, menthol was demonstrated to sustain specifically nicotine seeking behavior in rats, although this depended on the cooling effect (36). A further study by Biswas *et al.* (37) concluded that: "menthol enhances the reinforcing effects of nicotine, and the effect of menthol was specific to nicotine. These findings suggest that menthol directly facilitates nicotine consumption, thereby contributing to tobacco smoking". In addition, menthol was discussed to inhibit metabolism of nicotine (38), thus facilitating a higher systemic exposure.

A recent report suggests that menthol increases nicotine-induced dopamine release in the nucleus accumbens (39). This may indicate a synergistic rewarding effect of menthol on nicotine. A more detailed paper (40) indicates that menthol, unlike sucrose or saccharin, does not change phasic release of dopamine in the nucleus accumbens on its own. In addition, oral menthol, which does not alter intravenous nicotine self-administration, reverses oral nicotine taste aversion in a two-bottle choice test. However, Nesil *et al.* (41), concluded from a study in rats that "pharmacological interactions of menthol with nicotine reduce, rather than increase, nicotine's reinforcing effects and some measures of relapse vulnerability".

Altogether, except for Nesil *et al.* (41), it seems that there is an intricate interaction between nicotine and menthol which increases both addictiveness and attractiveness of tobacco by decreasing its aversive effects. This seems consistent with the conclusions by the FDA TPSAC in the comprehensive 2011 report on menthol (2), stating that there is sufficient evidence to indicate that those who smoke menthol tend to be more dependent. Moreover, menthol has been recently acknowledged as additive that can enhance nicotine dependence by a WHO/FCTC expert group, consulting on measures to reduce addictiveness of tobacco products (42).

5.11.9 Overall conclusion on additive

The industry evaluated menthol application levels of up to 1.8% and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. Importantly, much relevant data from independent literature was omitted. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports but especially using independent literature.

Menthol was applied up to 1.8% in tobacco, is a volatile compound with a transfer rate of 10 to 30%, and mostly stays intact during pyrolysis. Although previous studies reported that application of menthol leads to increase of furfural (Carc. 2), this has not been followed up by the industry. None of the reported pyrolysis products has CMR properties. However, new experiments have not been conducted. Also the industry's assessment of menthols influence on toxicity was insufficient. Important studies were excluded from the industry's assessment and indirect effects (e.g. due to larger puff volumes and increased pulmonary epithelium permeability to cigarette smoke toxicants) were not addressed. Therefore, a direct and especially indirect effect of menthol as an additive in cigarettes and RYO tobacco on toxicity of mainstream smoke cannot be ruled out.

The industry's assessment of inhalation facilitation and influence on addictiveness failed to acknowledge striking evidence for these effects. Firstly, menthol can facilitate inhalation in the context of cigarette smoking. This property of menthol in tobacco products has a high relevance for new and experimenting smokers but is less relevant for experienced and adapted smokers. Further, there is substantial evidence that menthol can increase the addictiveness of nicotine, although it remains problematic to demonstrate significant and measurable effects due to the lack of suitable and validated methods. Further, decreased aversion to tobacco smoke is regarded by the reviewers as a major mechanism to promote exposure to nicotine and thus to promote tobacco dependence. The industry proposed a content of 1.2% menthol to mark a threshold for characteristic flavor properties. However, this is not in agreement with the existing literature and not plausible according to the review panel.

The main concern of the review panel with regards to using menthol as an additive in cigarettes and roll your own tobacco is its physiological effect (TRPM8 activation) at even low levels that do not lead to a characterizing flavor. This physiological effect leads to inhalation facilitation, increase of addictiveness of nicotine, and indirectly to an increased toxicity.

Since May 2020, cigarettes and RYO products containing menthol as a characterizing flavor are prohibited based on the Tobacco Products Directive (TPD art. 7.1). However, the TRPM8-dependent cooling effect -that facilitates inhalation of irritating fumes- occurs already at levels far below the threshold of characteristic aroma properties. Importantly, this is an intrinsic property of menthol and does not comply with Article 7 (6d) of the TPD, even if application levels in tobacco might not induce measurable effects. Therefore prohibiting the addition of menthol at all application levels is advised. Some member states, such as Germany and Finland, currently prohibit application of menthol at any

application level based on its inhalation facilitating properties.

The industry report on menthol further illustrates that regulators cannot expect to be made aware of relevant issues and health risks by the tobacco industry, regardless of whether formal requirements of TPD Article 6 are sufficiently met.

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5.12 Report of Propylene Glycol

5.12.1 Abstract

Propylene glycol is an odorless liquid. It has been widely used for years in food industry, pharmaceuticals, cosmetics, paint, plastic, and to create artificial smoke. In tobacco, propylene glycol is used as a humectant in application levels up to 5.0%. In the industry report three levels are tested 2.5% (Low) 5.0% (Max) and 6.0% (Max-plus) in test cigarettes. Propylene glycol is a non-volatile compound in ambient conditions. Transfer rates below 1% were reported in the current industry experiments, while previous publications report of 7.3-8.0%. During pyrolysis, propylene glycol transfers mostly intact. Concerning smoke chemistry, a decrease of *m+p* cresol and phenol, and an increase of propylene glycol was described as a significant "overall effect" by the industry. The industry concluded in their report that application of propylene glycol did not result in significant effects regarding toxicity, addictiveness and inhalation facilitation. The industry report did not assess whether propylene glycol has a characterizing flavor. The review panel concluded that there

were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness and inhalation facilitation. Importantly, the chemical comparative analysis lacked an appropriate control; a test cigarette without a humectant was used as a reference resulting in altered combustion conditions likely to affect the levels of toxicants in mainstream smoke. Moreover, (characterizing) flavoring properties were not assessed. The main concerns of the review panel with regards to the assessment of propylene glycol as an additive in cigarettes and roll your own tobacco are its unclarified influence on smoke chemistry, the possible CMR properties of pyrolysis products (e.g. pyruvaldehyde; Muta. 2) and the possible impact on the attractiveness.

5.12.2 Background

Propylene glycol is used as a humectant; it helps to keep tobacco moist and is added during the casing process. It is added frequently and in relatively high amounts (averaged: 1.636 weight percent). Previous assessments of scientific data on propylene glycol as an additive in tobacco products have identified several concerns (1, 2).

Regarding toxicity: Trace amounts of propylene oxide are present in the additive, which is considered a possible human carcinogen. In addition, the pyrolysis of propylene glycol may lead to the formation of 1,3-propylene glycol, acetol, acetic anhydride, and pyruvaldehyde. Furthermore, SCENIHR estimates that the reaction between propylene glycol or its pyrolysis products and the thousands of other substances present in cigarette smoke can lead to additive effects and reactions.

Regarding attractiveness: Internal tobacco industry documents reported that adding 3 – 7 weight percent of propylene glycol increased the mildness of mainstream smoke and reduced irritation. As humectants are added to trap water, it can positively influence the attractiveness by improving the palatability of cigarettes, making them more appealing and easier to smoke.

Regarding Addictiveness: There are currently no data to suggest an addictive effect of propylene glycol.

Regarding characterizing flavor: Propylene glycol is not expected to have a characterizing flavor in its unburnt form.

5.12.3 Literature review

The industry report provides two literature overviews for propylene glycol, one regarding additive in general and a second one regarding propylene glycol when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4). For example, some important sources were not used (i.e. ECHA). Relevant issues, such as physiological functions/properties or effects by inhalation are not sufficiently covered. Also no information on toxicological effects of pyrolysis products were included.

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.12.4 Chemistry and Pyrolysis products

The report provides a brief description of propylene glycol (CAS 57-55-6), summarizing chemical

characteristics and lot properties. The submitted data covers information on the manufacturer and compliance with applicable EU regulations.

Application levels: Propylene glycol levels applied in test cigarettes varied between 2.5% and 6% (target levels Low 2.5%; Max, 5%; Max-plus 6%; with actually achieved concentrations of 2.05%, 4.53%, and 4.80%, respectively.

Transfer of propylene glycol in mainstream smoke: Transfer rates for propylene glycol were found to be below 1%. As acknowledged in the report, previous studies (3), demonstrated much higher transfer rates of 7.3 – 8.8%. As a possible explanation, it was proposed in the report that the filter ventilation of the current test cigarettes influences the transfer of some tobacco constituents and/or additives. Notably, this argument raises principal concerns, whether filter ventilation can affect the relevance of the submitted data. To clarify these findings, additional data might be necessary, such as from supplemental experiments using an intense smoking regime with blocked filter ventilation.

Pyrolysis experiments: The industry report summarizes previous pyrolysis studies. Baker and Bishop (4) demonstrated that 1,2-propylene glycol is not undergoing excessive pyrolysis, since 86.3% of the applied compound remained intact. Identified pyrolysis products or modified products include 1,3-propylene glycol (6.2%), acetol or acetic anhydride (4.7%), and pyruvaldehyde (2.8%).

Chemical analysis of mainstream smoke: For comparative testing of mainstream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (2.5 - 6%). The industry report refers to the studies of Garworski *et al.* (5), Baker *et al.* (4), Carmines *et al.* (6) and Coggins *et al.* (7). The first study found mildly decreased values for nicotine and some other compounds, but the other three report an increase in several compounds, such as carbonyls, HCN, CO, NH3, Pb and several hydrocarbons and PAH.

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, are included in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F). In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

Further, the propylene glycol-free control cigarettes lack propylene glycol, but also any other humectant. This might affect moisture, burning properties and conditions and the composition of tar, perhaps leading to modified toxicant levels not attributable to specific properties of propylene glycol, but due to the modified combustion conditions. Not even a "Low" application level of glycerol was applied as an alternative humectant, which is regarded as a notable methodological mistake, neither the comparison with another humectant. The review panel questions, whether the "additive-free" reference cigarette can be regarded as an appropriate control. Such a product represents a

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

faulty manufactured cigarette that is lacking an important technical feature, and is not likely to be placed on the market. Humectants are technical compounds that are required to manufacture tobacco products. Therefore, the industry should have analyzed whether propylene glycol can be regarded as comparatively safe option or whether other applicable humectants bear lower risks. The toxic properties of propylene glycol should have been studied and summarized in relation to glycerol and other compounds that are used as humectants in tobacco. A major shortcoming in the industry report is the lack of technical background information on the selection and application of humectants. This should include a summary of distinct effects of propylene glycol, glycerol and other humectants on the relevant properties of tobacco products.

In the comparative experiments performed by the industry, application of propylene glycol at "Max-plus" level resulted in decreased *m+p* cresol and phenol that exceeded the variability of the 3R4F monitor cigarette and was regarded as a statistically significant "overall effect". Further, increased levels of carbonyls, cadmium, NO, and NOx were found in the smoke of cigarettes containing propylene glycol, although these were not regarded to be significant by the industry. It is somehow surprising that the content of water is lower in relation to the additive free control that contains no humectants, except a minute amount of endogenous glycerol (0.06 mg/cigarette). This is a marked contrast to the glycerol test cigarettes that showed a strong glycerol-related increase of water in MSS. Also, the water content in the smoke of glycerol-cigarettes was up to 10-fold higher than in the propylene glycol samples. These differences might be attributable to experimental variation, but they probably indicate an overall modification of combustion process and should be discussed and explained in more detail.

Since it is known that aldehydes may inhibit monoamine oxidases (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). As the data was of poor quality, it was difficult to draw conclusions on the influence of propylene glycol. Although there were increased levels of carbonyl formation, the review panel concluded that these were not attributable to propylene glycol, as the compound levels did not increase with increasing levels of propylene glycol in the test cigarette.

Evaluation by the review panel: The submitted report on propylene glycol examines the transfer rate, pyrolysis and the levels of harmful and potentially harmful constituents in cigarette smoke. However, no new pyrolysis studies were performed and there are limitations in the comparative testing approach. Humectants are major additives that are applied at levels of 5% or more. It is essential to have reliable data on these compounds. Taken together, there are conceptual and technical shortcomings in the data on smoke chemistry. To address these issues appropriately, the industry should comprehensively summarize effect of humectants on toxicant levels and on other relevant properties of cigarettes, besides providing data on specific compounds. Shortcomings in the industry's approach and methodology to assess smoke chemistry limit the usefulness of provided data. Although the data provided by the industry do not suggest an increase of toxic compounds due to combustion of propylene glycol, the provided experiments are not sufficient to determine whether propylene glycol affects smoke chemistry.

5.12.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – ASSESSSED

As discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relatively extensive evaluation of the toxicity of propylene glycol when ingested. Several LD50 have been determined in several species: 24900 mg/kg body weight (bw) in the mouse,

22000 mg/kg bw in the rat, 180000 mg/kg bw in the rabbit, 19700 mg/kg bw in the guinea pig and 20000mg/kg bw in the dog. In the OECD report published in 2001, the authors concluded that propylene glycol is very well tolerated by rats and dogs after repeated ingestion (8). EFSA (9) and the JECFA (10) established an Acceptable Daily Intake of 0 – 25 mg/kg bw/day. Slight decreases in hemoglobin, hematocrit and erythrocytes and a slight increase in bilirubin was seen at the highest dose (5000 mg/kg bw) in a chronic study of 2 years in Beagle dogs (11). No carcinogenic effects have been shown in a long term dietary study in rats (12). In an NTP continuous breeding study in mice, the author concluded that propylene glycol is not a reproductive toxicant in males or females or in their progeny (13). In a prenatal developmental toxicity study in mice, Wistar rats, golden hamsters and Dutch-belted rabbits, neither developmental nor maternal toxicity was detected at the highest dose (up to 1600 mg/kg bw/day) (13). Nevertheless, exposure from the diet is completely different from exposure by inhalation, and such effects are not representative for the inhalation route. Therefore, it is not relevant to use the assessment that was conducted for the use of propylene glycol as a food additive to evaluate its toxicity when included in tobacco products.

B. Evaluation of **additive itself (inhalation)** – PARTLY ASSESSED

In the literature review, the industry identified and summarized several inhalation studies. A 28 days inhalation study in rats showed some clinical signs of ocular and nasal irritation (14). In the same study, multiple decreases in blood parameters were described in the two highest exposure groups in female dogs. A decrease of mean body weight of female rats was observed in another study (15). However, no inhalation studies addressing carcinogenicity and reproductive toxicity are mentioned in the manufacturers report (16-18).

C. Evaluation of the **pyrolysis products** – NOT ASSESSED

The industry report does not evaluate the toxicity of the pyrolysis products. Three substances were identified in the report: 1,3-propylene glycol, acetol or acetic anhydride and pyruvaldehyde. Moreover, the identification of pyrolysis products was based on the Hoffmann list, which is known to be out of date. Thus, it is possible that some potentially toxic compounds resulting from pyrolysis of propylene glycol were not identified. Based on assessment by the review panel (see Annex III – "Pyrolysis product table") it was concluded that one of the reported pyrolysis products may have CMR properties (Pyruvaldehyde, Muta. 2).

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

For comparative testing, the report includes both a literature review and new studies. The included *in vivo* tests showed a statistical difference on one parameter, namely a 13 – 15% increase of carboxyhemoglobin during exposure to cigarette smoke (19). The industry concludes that the newly performed experiments and the reviewed literature does not suggest that propylene glycol in mainstream smoke could have CMR properties or other toxic effects.

However, the review panel questions the validity of this conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). Similarly, the lack of an appropriate control also affects the interpretation of the toxicity comparative testing. In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 2.5-6 % propylene glycol does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. In contrast, the members of the review panel conclude that the presented toxicological assessment of propylene glycol is insufficient to reach a conclusion regarding the toxicity of propylene glycol when used as a tobacco additive and humectant. There are no data on inhalation toxicity of the pyrolysis

products of propylene glycol and there were several methodological limitations in the comparative testing approach. Additionally, the pyrolysis product pyruvaldehyde (Muta. 2) was not included in the smoke chemistry analysis. Thus, a contribution to CMR properties due to application of propylene glycol as an additive in cigarettes and RYO tobacco cannot be ruled out.

5.12.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: No concerns were identified regarding possible addictive effects of propylene glycol or its pyrolysis products.

Inhalation facilitation: Propylene glycol is added to tobacco as a humectant, to keep moisture in tobacco and prevent it from drying out. This humidification reduces the harshness of smoke and may thereby facilitate inhalation (2).

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of propylene glycol, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added propylene glycol to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of propylene glycol on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from propylene glycol. There were also no studies reported assessing the effects of propylene glycol on nicotine bio-availability and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores.*

Evaluation by the review panel: The provided data show no effect of adding propylene glycol on inhalation facilitation and nicotine uptake, but these data are limited and do not sufficiently address the previously identified concern regarding humidification. Altogether, there is insufficient evidence to rule out an influence on inhalation facilitation due to application of propylene glycol as an additive in cigarettes and RYO tobacco.

5.12.7 Characterizing flavor

Whether the additive propylene glycol can be responsible for introducing a characterizing flavor or odor to tobacco products has not been addressed in the industry report. Propylene glycol is not known to be a flavoring compound, and therefore no such effect is expected. However, the change of combustion conditions can lead to other products that may have flavoring properties. This is not assessed by the industry.

5.12.8 Overall conclusion on additive

The industry evaluated propylene glycol application levels of up to 6 %, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. In spite of the before mentioned limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Propylene glycol is a non-volatile humectant that transfers mostly intact in low rates. Propylene glycol has a large influence on smoke chemistry that was not assessed adequately by the industry, since a suitable reference cigarette (with a humectant) was not included in the analysis. As humectants are technically necessary compounds, the influence of propylene glycol on chemistry and toxicity should have been assessed in comparison to cigarettes containing other humectants that could be applied instead of propylene glycol. Inhalation toxicity was assessed in terms of a literature review. However, the available evidence is not sufficient to rule out any increase in toxicity. Influence on inhalation facilitation and addictiveness have not been assessed sufficiently. Propylene glycol is a humectant that keeps the tobacco moist and thus increases palatability of the product. No assessment has been performed regarding the influence of this additive on attractiveness.

The main concerns of the review panel with regards to the assessment of propylene glycol as an additive in cigarettes and roll your own tobacco are the yet unclarified influence on smoke chemistry, the possible CMR properties of pyrolysis products (e.g. pyruvaldehyde; Muta. 2) and the possible impact on attractiveness.

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5.13 Report of Sorbitol

5.13.1 Abstract

Sorbitol is a sugar alcohol that is widely used as emulsifier, sugar substitute or humectant in food, cosmetics and health care products. In tobacco products, sorbitol is applied up to 1.2 %. In the industry report three levels are tested 0.6% (Low) 1.2% (Max) and 1.8% (Max-plus). Sorbitol is a non-volatile compound and pyrolyzes completely, therefore no transfer rates have been determined. Some of the reported pyrolysis products (e.g. furfural, Carc. 2; furfuryl alcohol, Carc. 2) have CMR properties. Concerning smoke chemistry, an increase of acrolein, formaldehyde and other carbonyls was reported by the industry, while they concluded in their report that application of sorbitol did not result in significant effects regarding toxicity, addictiveness and inhalation facilitation. Furthermore, it was not assessed whether sorbitol has a characterizing flavor. The review panel concluded that there were limitations in the overall approach and applied methodology (see Chapter 4), due to which the presented data did not allow for a complete interpretation of chemical comparative emission testing, toxicity, addictiveness and inhalation facilitation. Moreover, (characterizing) flavoring properties of sorbitol were not assessed. Increase in formaldehyde and acrolein is seen as significant and relevant by the review panel, and increases of other carbonyl compounds seem to be relevant as well, but the poor quality of data hampers data interpretation. Furthermore, an increase in cadmium was found. The main concerns of the review panel with regards to using sorbitol as an additive in cigarettes and roll your own tobacco are the carcinogenicity of pyrolysis products (furfural, furfuryl alcohol), the increase of toxic compounds (especially formaldehyde and acrolein) in the emissions and the possible enhancement of addictiveness due to increased emission of aldehydes that are known precursors of MAO inhibitors.

5.13.2 Background

Sorbitol (CAS 50-70-4) is a sugar alcohol that is widely used as emulsifier, sugar substitute or humectant in food, cosmetics, and health care products. Sorbitol plays a major role as additive in cigarettes and roll-your-own tobacco products. A maximum application level of 1.2% was reported by industry.

Previous assessments of scientific data on sorbitol as an additive in tobacco products have identified several concerns (1):

Regarding toxicity: During pyrolysis of sorbitol, carcinogenic (e.g. formaldehyde), possibly carcinogenic (e.g. acetaldehyde, furfural) and other toxic compounds are formed.

Regarding addictiveness: Combustion products of sorbitol (e.g. acetaldehyde, formaldehyde) were proposed to increase the addictive effect of nicotine through the process of MAO inhibition.

Regarding attractiveness: As sorbitol is added as a humectant, to trap water, it can positively influence the attractiveness by improving the palatability of cigarettes, making them more appealing and easier to smoke.

5.13.3 Literature review

The industry report provides two literature overviews for sorbitol, one regarding sorbitol in general and a second one regarding sorbitol when applied as a tobacco additive. Several shortcomings in the literature search were identified by the review panel, such as an underrepresentation of independent studies and a lack of inclusion of several relevant topics in the literature search, such as the inhalation toxicity, respiratory sensitization and toxicity or addictiveness of pyrolysis products (see Section 4.4). In addition, some important relevant sources were not used (i.e. ECHA).

Some findings and shortcomings of the industry's literature search are discussed below in the according sections or at the end of the report. Taken together, the literature overview provided is biased and incomplete. This limits its usefulness for risk assessment, and represents a major limitation of the industry report.

5.13.4 Chemistry and Pyrolysis products

The report provides a description of sorbitol as additive, including specification for the substance used to manufacture the cigarettes that were analyzed in the comparative testing program. Information on the supplier, lot-specification, purity and compliance with the relevant European regulation has been provided. The report states that the referenced lot represents the typical characteristics of the additive sorbitol. It is used as a syrup that contains 77.7 % sorbitol dissolved in water.

Application levels: In tobacco products, sorbitol is applied up to a maximum level of 1.2 % (12 g per kg tobacco) by the reporting companies. Notably, the report does not provide information on maximum application levels for sorbitol, but only on current products of the reporting companies. Consequently, higher levels (>1.2%) might be applied by other manufacturers. The sorbitol levels applied in test cigarettes varied between 0.6 and 1.8% (target levels Low 0.6%; Max 1.2%; Max-plus 1.8%; actually achieved levels of 0.65%, 1.1%, and 1.6%, respectively).

Transfer of sorbitol into mainstream smoke: Based on the non-volatile nature of sorbitol, the industry stated that it is unlikely to transfer intact. However, no new experiments were perfomed to confirm this. Further, the industry stated that no published studies evaluated transfer of sorbitol into mainstream smoke.

Pyrolysis experiments: The industry refers to studies by Baker *et al.* (2), Coggins *et al.* (3) and Gomez-Siurana *et al.* (4), that analyzed pyrolysis and smoke chemistry. Sorbitol was reported to

undergo complete pyrolysis, forming mainly furfural. Baker and Bishop (2) had identified the following pattern of decomposition products under pyrolytic conditions: 31.4% furfural, 9.7% propylfuran, 7.7% acetylfuran, 6.4% furanone, 5.2% methoxycyclopentenone and others. No additional experiments have been performed on transfer and pyrolysis and no new data are submitted.

Chemical analysis of mainstream smoke: For comparative testing of mainstream smoke chemistry, the report includes both a literature review and new studies. All three application levels were included in the new experiments (0.6 – 1.8%).

The limitations of the comparative testing approach and statistical methodology applied in the industry reports, as identified by the review panel members, are described in Chapter 4. In short, the newly performed industry experiments only included the ISO smoke generation method, which is known to result in levels below real-life exposure. This may contribute to an underestimation of the content of chemical compounds. Although the selection of compounds included in the chemical analysis was based on the WHO list recommended by SCENIHR, this list was not extended with other pyrolysis products of the additives. Thus, possible significant contributions to smoke chemistry by some of the pyrolysis products was not assessed. For the statistical testing, the difference between test cigarettes with and without the additive in the emissions of each chemical compound was compared with the variability of these compounds in an additive free reference cigarette (3R4F) . In this analysis, historical data from several laboratories were used to determine the variability for the reference cigarette, an approach seldom applied in other types of scientific studies. This leads to an overestimation of the variation that can be expected within the study itself, and may cause false negative results. Also, a 99% confidence criterion was applied in the industry reports, in contrast to the 95% criterion commonly used in scientific literature. Finally, the evaluation by the industry involved several different statistical analyses on the same data, reporting only findings that passed all the individual significance tests, rather than reporting all significant findings. These choices are also likely to contribute to false negatives.*

All analyzed carbonyl compounds (acetaldehyde, acetone, acrolein, butyraldehyde, crotonaldehyde, formaldehyde, propionaldehyde) and cadmium were increased in the mainstream smoke in a dose-dependent manner. All increases exceeded the variability of the 3R4F reference cigarette at "Max-plus" application levels and for some compounds also at lower levels. In the industry report, statistically significant and consistent increases in relation to the additive have only been acknowledged for formaldehyde and acrolein. In contrast, the substantial increases of other carbonyls, such as acetaldehyde, are hardly discussed.

Since it is known that aldehydes may inhibit monoamine oxidase (MAO) and thereby increase tobacco addictiveness, the review panel re-evaluated the comparative testing results for carbonyl compounds presented by the industry (see Section 3.4 and Annex II). Despite the poor quality of data, some information could be extracted. Almost all carbonyls increase with sorbitol concentrations in an additive level related manner. The increase in formaldehyde and acrolein is seen as significant and relevant. The increase of other carbonyl compounds seem to be relevant as well, but the poor quality of data (see Chapter 4) hampers data interpretation. Furthermore, the observed increase of cadmium has not been further assessed or explained. In their literature review, the industry denied relevant effects of sorbitol on the levels of carbonyls in tobacco smoke. These arguments were based on previous literature (3, 5) while some of their own presented experimental findings were not fully taken into account in the discussion.

Evaluation by the review panel: The submitted report on sorbitol examines the pyrolysis, as well as the levels of harmful and potentially harmful constituents in cigarette smoke, however, not all aspects of smoke chemistry are covered comprehensively. Sorbitol was reported to undergo complete pyrolysis,

* A false negative result means that the statistical test indicates no change in for instance chemical composition, when in reality there is a change.

forming mainly furfural. Several other compounds have been identified in pyrolytic experiments, such as propylfuran and acetylfuran. None of the putative decomposition products have been included in the comparative analysis of smoke chemistry. Addition of sorbitol to the Max-plus level (1.8%) led to profound increases for all carbonylic compounds. Compared to the sorbitol-free reference cigarette, variations of individual carbonylic compounds ranged between 34.5% (butyraldehyde) and 102% (formaldehyde) at the Max-plus application level. The industry acknowledged significant and meaningful increases for formaldehyde and acrolein, but failed to discuss the substantial variations of other relevant aldehydes and in cadmium. In spite of the poor quality of the provided data, the members of the review panel conclude that there may be an influence on smoke chemistry when sorbitol is applied as an additive in cigarettes and RYO tobacco at tested levels.

Further, sorbitol could be used in combination with carbohydrates to adjust aldehyde levels in the smoke of individual cigarette brands. The interplay between sorbitol, starches and other sugar supplements on aldehyde levels, as well as putative effects on addictiveness, need to be further explored.

5.13.5 Toxicity and CMR properties

As specified in Chapter 3 and 4, there are four main strategies for toxicological evaluation, these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – PARTLY ASSESSSED

As discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. However, the industry report includes a relative extensive evaluation of the toxicity of sorbitol when ingested. The report provided by the industry is almost exclusively based on conclusions of the JECFA panel (1978 and 1982) (6, 7). Based on these, industry considers sorbitol to be non-genotoxic, non-mutagenic, and non-carcinogenic.

B. Evaluation of **additive itself (inhalation)** – NOT ASSESSED

The inhalation toxicity of sorbitol is not evaluated in the industry report. Literature on sorbitol toxicity by inhalation seems limited. According to the industry report, the pyrolysis studies have shown that sorbitol undergoes pyrolysis when burnt, and sorbitol will not transfer intact, as it is a non-volatile compound.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

The pyrolysis products identified in the report were not evaluated in terms of oral nor inhalation toxicity. Moreover, the identification of pyrolysis products was based on literature applying the Hoffmann list, which is known to be out of date. Thus, it is possible that some known potentially toxic compounds resulting from pyrolysis of sorbitol were not determined. . Moreover, The industry report does not evaluate the toxicity of pyrolysis products. Based on assessment by the review panel (see Annex III – Pyrolysis product table) it was concluded that some of the reported pyrolysis products (e.g. furfural, Carc. 2; furfuryl alcohol, Carc. 2) have CMR properties. In addition, a mutagenicic effect of pyruvaldehyde has been suggested, but evidence for this in human studies was not conclusive (IARC 3 classification).

D. Evaluation of **mainstream smoke (comparative testing)** – PARTLY ASSESSED

Based on the performed comparative *in vitro* experiments that did not identify significant effects, the industry report concludes that an inclusion of sorbitol in tobacco does not increase the CMR properties of the mainstream smoke. However, as discussed in the Chemistry section, sorbitol increases the formation of formaldehyde and acrolein (+102% and +81%, respectively). Formaldehyde is a substance classified as a CMR carcinogen cat. 1B and mutagen 2 under the EC Regulation No

1272/2008.

However, the review panel questions the validity of the industry conclusion due to limitations in the underlying studies. The limitations of the comparative testing approach described in Section 4 regarding smoke generation methods and statistical analysis, also concern the toxicity data (see also Chapter 4). In addition, the in vitro tests included in the newly performed industry studies are not sufficient to perform an evaluation of the CMR properties, since in vivo studies are required to address this issue. Nevertheless, the review panel acknowledges that new in vivo studies regarding tobacco products are neither appropriate nor allowed for ethical reasons.

Evaluation by the review panel: The industry concluded that inclusion of 0.6-1.8% sorbitol does not increase the toxicity of cigarettes or RYO tobacco to a significant or measurable degree. However, in their evaluation the review panel concluded that the presented toxicological assessment of sorbitol is insufficient. There were several methodological limitations in the comparative testing approach. The chemistry results of the comparative studies showing increased emissions of several compounds of toxicological concern (such as formaldehyde and acrolein) were not followed up with toxicological assessments. Furthermore, in the comparative toxicity experiments only CMR properties were considered and no data was presented on the inhalation toxicity of sorbitol and its pyrolysis products. Some of the reported pyrolysis products (furfural, Carc. 2; furfuryl alcohol, Carc. 2) have CMR properties. Their levels in mainstream smoke have not been assessed in the chemical analysis of mainstream smoke. Overall, a contribution to increased CMR properties upon application of sorbitol as an additive cannot be ruled out.

5.13.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness: Pyrolysis of sorbitol has been shown to lead to the formation of acetaldehyde and other aldehydes, which have the potential to increase the addictiveness of cigarette smoke via Monoamine Oxidase (MAO) inhibition (8, 9).

Inhalation facilitation: Sorbitol is added to tobacco as a humectant, to keep moisture in tobacco and prevent it from drying out. This humidification reduces the harshness of smoke and may thereby facilitate inhalation (1).

Industry experiments: *As an indirect estimate of inhalation facilitation and nicotine uptake properties of sorbitol, plasma pharmacokinetics of nicotine as well as several smoking behavior parameters such as puff duration and volume and inhalation depth and volume, were measured and described in the industry report. However, only descriptive statistics were provided, and no statistical test to compare the test cigarette with added sorbitol to the additive free reference cigarette was performed. The industry concludes based on their studies that there is no effect of sorbitol on inhalation facilitation. Although the reported differences are small, it is not possible to verify this conclusion without statistical tests. In spite of the previously identified concerns, no experimental tests on inhalation facilitation and nicotine uptake were reported for any metabolites or pyrolysis products from sorbitol. Specifically, none of the reported studies did asses the capacity of metabolites and pyrolysis products of sorbitol on monoamine oxidase inhibition. There were also no studies reported assessing the effects of sorbitol on nicotine bio-availability and clinical markers of nicotine addiction, such as craving, withdrawal symptoms or dependence scores.*

Evaluation of the review panel: The provided data show increased levels of formaldehyde and other carbonyls in mainstream smoke of cigarettes containing sorbitol, which raises concern regarding addictiveness. The provided clinical tests show no effect of adding sorbitol on inhalation facilitation and nicotine uptake, but these data are limited and do not address the previously identified concerns regarding MAO inhibition and humidification. In the independent re-evaluation of the of mainstream smoke data reported in the industry report (see Section 4 of this Chapter and Annex II), almost all

carbonyl concentrations increased with sorbitol, while the increase in formaldehyde (and acrolein) was evaluated as significant and relevant by the review panel. Other compounds that might be relevant for inhalation facilitation or nicotine delivery were not assessed in the industry report. Altogether, an influence on addictiveness or inhalation facilitation due to addition of sorbitol to tobacco cannot be ruled out.

5.13.7 Characterizing flavor

Whether the additive sorbitol can be responsible for introducing a characterizing flavor or odor to tobacco products has not been addressed in the industry report. Sorbitol itself is not likely to lead to a characterizing flavor or odor in tobacco. However, combustion of sugars and sugar alcohols like sorbitol is known to contribute to complex aromas. It is also not clear to what extent pyrolysis products of sorbitol, such as furfural (10), can contribute to a (characterizing) flavor and/or odor in burned tobacco. However, even in the absence of a characterizing flavor, these flavoring compounds singly or in combination with other smoke constituents may contribute to the attractiveness of smoking by improving smoke flavor or odor and masking its bitter taste. It is a notable shortcoming that these effects of sorbitol and its pyrolysis products were not assessed.

5.13.8 Overall conclusion on additive

The industry evaluated sorbitol application levels of up to 1.8%, and concluded that there was no risk associated with its application as an additive in cigarettes and RYO tobacco in terms of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor. In the evaluation of the industry report, the review panel concluded that there were clear shortcomings in the approach and methodology applied in the submitted industry report. The industry's assessment of toxicity, addictiveness, inhalation facilitation, nicotine uptake, and characterizing flavor was evaluated as insufficient. In spite of these limitations, the review panel could draw some conclusions based on the information provided in the industry reports and independent literature.

Sorbitol is a non-volatile compound that undergoes pyrolysis. Although no new pyrolysis experiments were performed, some of the listed pyrolysis products (furfural, Carc. 2; furfuryl alcohol, Carc. 2) have CMR properties. These classified carcinogens were not included in the chemical comparative experiments. Application of sorbitol in test cigarettes caused an additive-level related increase of carbonyl compounds, especially for formaldehyde and acrolein, and cadmium in the chemical analysis of mainstream smoke. As formaldehyde is classified as a carcinogen and mutagen, the review panel concludes that addition of sorbitol contributes to the CMR properties of the mainstream smoke. Inhalation toxicity was not evaluated in the industry report. Thus, given the application level of up to 1.8% and based on the presented data, it can be concluded that use of sorbitol as an additive in cigarettes and RYO tobacco is with concern regarding toxicity.

The influence on inhalation facilitation and addictiveness have not been assessed adequately. Aldehydes that are known MAO inhibitors were increased at tested levels raising concerns regarding addictiveness. The impact of sorbitol on tobacco flavor has not been addressed in the industry report. However, pyrolysis of sorbitol is known to cause formation of flavoring compounds, which may improve smoke flavor and thereby increase the attractiveness of tobacco products.

The main concerns of the review panel with regards to using sorbitol as an additive in cigarettes and roll your own tobacco are the carcinogenicity of pyrolysis products (furfural, furfuryl alcohol), the increase of toxic compounds (especially formaldehyde and acrolein) in the emissions, and the possible enhancement of addictiveness due to increased emission of aldehydes that are known MAO inhibitors.

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5.14 Report of Titanium dioxide

5.14.1 Abstract

Titanium dioxide is applied as a white pigment to various consumer products such as paint, sun screen, toothpaste, paper and plastics. Titanium dioxide is a non-volatile compound. It is used as a whitening agent in cigarette filters (0.5 mg/cig filter), where it is bound to the filter material. It was stated by the industry, that carcinogenicity would only be of principle concern when titanium dioxide was released out of the filter. Therefore, a sham smoking experiment has been performed with unlit reference cigarettes containing 0.5 mg titanium dioxide per cigarette filter. No significant release of respirable titanium dioxide particles was observed. However, this set-up is seen as insufficient by the review panel and transfer into smoke cannot be ruled out. Further, smoke chemistry, addictiveness, inhalation facilitation or characterizing flavor were not assessed in the industry report. The review panel concluded that the industry did not provide sufficient data on titanium dioxide as an additive to cigarettes to fulfill requirements by TPD. Also, products that contain titanium dioxide in tobacco, in any other component than the filler, including cigarette paper, or in higher amounts than 0.5 mg are not covered by the submitted report and do not comply with Article 6 of the TPD. In addition, the

European Commission has classified titanium dioxide as a "carcinogen category 2", the classification becomes effective on September 9th, 2021. Since it cannot be concluded that there are no titanium dioxide particles in the smoke without performing combustion experiments, from that date, tobacco and related products containing titanium dioxide do not appear to comply with Article 7 (6e) of the TPD.

5.14.2 Background

Titanium dioxide (TiO2) is applied as a white pigment to various consumer products such as paint, sun screen, toothpaste, paper and plastics. It is used as a whitening agent in cigarette filters, where it is bound to the filter material. It has also been reported as an ingredient of filter paper inks tipping paper and tipping inks. The industry stated in their report that titanium dioxide is neither mechanically nor thermally released from the cigarette filter though this assertion is not substantiated. Previous assessments of scientific data on titanium dioxide as an additive in tobacco products have identified several concerns (1).

Regarding toxicity: Nano-size titanium dioxide induce an acute inflammation in the lungs, that may be reversible depending on the dose and the time after exposure. Both nano and non-nanosize titanium dioxide was classified by IARC as a Group 2B carcinogen (possibly carcinogenic to humans) (2).By the time the industry report was submitted, the CLP classification of titanium dioxide was still in progress. In the meantime, the European Commission has classified titanium dioxide as a "carcinogen category 2", the classification becomes effective on September 9th, 2021 (3). From that date, tobacco and related products containing titanium dioxide do not comply with Article 7 (6e) of the TPD: "Member States shall prohibit the placing on the market of tobacco products containing the following additives: (a)……. (e) additives that have CMR properties in unburnt form." .

Other toxicities than pulmonary effects have been reported following exposure to titanium dioxide as well: cardiotoxicity, immunotoxicity, neurotoxicity, liver and kidney toxicity (see for example ANSES report (4) for detailed reporting). Mutagenicity and reprotoxicity are still debated.

5.14.3 Literature review

Only one literature study was included in the literature review, for the toxicological assessment of titanium dioxide as a tobacco additive. This was an industry study in which no associations between occupational exposures to titanium dioxide and cancer risks were confirmed (5). Two publications reporting lung tumors in rats after chronic inhalation of titanium dioxide were dismissed for reasons of inter-species variability (6, 7). It was hypothesized that the observed lung tumors could be due to lung overload, as the rat is known for its particular pulmonary sensitivity (see industry report page 4).

Considering the toxicity concerns mentioned above (ECHA RAC (8)), the review panel concludes that the literature overview provided is biased and incomplete. Although some studies used in this opinion are also mentioned in the industry report, the RAC opinion itself is hardly quoted and discussed in the industry report. This limits the usefulness of the industry report for risk assessment, and represents a major limitation.

5.14.4 Chemistry and Pyrolysis products

The industry report did not include the same test battery for titanium dioxide as for the other compounds. Instead, only one experimental study was performed to determine the release of titanium dioxide particles from the filter from unlit cigarettes with a sham smoking experiment.

Application levels: Application level in the tested product was 0.5 mg per cigarette filter. The industry report states that titanium dioxide is only used in cigarette filters.

Transfer of titanium dioxide into mainstream smoke: It is claimed in the report that "titanium dioxide is bound to the cigarette filter material in a manner that the titanium dioxide should not be mechanically released", but no further details are given in the text. Importantly, there are no references or specifications on the applied principle of mechanical or chemical conjugation. To address transfer of titanium dioxide into mainstream smoke, a sham smoking experiment was conducted by the industry and provided in the report. According to the report, an Imperial Tobacco in-house method was used to count and characterize particles released from cigarette filters. The additive-free reference cigarette that was used as a reference in the other additive reports contained 0.5 mg titanium dioxide per cigarette filter. It was sham smoked (meaning the cigarette was not lit) in a smoking machine under ISO conditions. Air at ambient temperature was drawn through the unlit cigarettes. Samples were collected on a gold membrane and optical microscopy was used to count and analyze size and shape. Air blank samples were used as a reference.

In the review panel's opinion, there may be methodological problems (not discussed in the industry report) to distinguish titanium dioxide particles and other particulate matter in MSS or Environmental Tobacco Smoke (ETS). Furthermore, sham smoking is a very limited approximation of cigarette smoking at the stage of consumption. These limitations should have been discussed in more detail in the industry report. Importantly, the industry should consider further analysis of whether the conditions (such as temperature or smoke) of a burning cigarette can enhance the release of titanium dioxide particles. It should also be clarified, whether the tested batches did contain nanoscale titanium dioxide or any specific form (crystallinity, shape, size, coating,…) of titanium dioxide. Moreover, the emissions of titanium dioxide generated from MSS can be impacted from the granulometry of filter titanium dioxide, or the procedure of its application on the filter. These might require new experimental approaches and additional work. However, the above important points need to be addressed, even if no standardized methods are yet available.

Pyrolysis experiments: Industry stated that titanium dioxide is not affected by combustion processes of tobacco or cigarette paper. Thus, the fate of titanium dioxide under pyrolysis conditions was not addressed.

Chemical analysis of mainstream smoke: As titanium dioxide is not undergoing combustion, industry did not investigate pyrolysis or smoke chemistry. Although assessments of pyrolysis would hardly be expected to provide useful information (if we consider that all the forms of titanium dioxide used are constituted of titanium dioxide only, i.e. no titanium dioxide coated with organic substances), it remains possible that titanium dioxide catalyzes chemical processes when the mainstream smoke (MSS) is passing through the filter. As comprehensive studies are requested, data on smoke chemistry should be provided. Ideally, this would involve filters that do not contain titanium dioxide as a reference. Analysis could include corresponding cigarettes without filters to confirm that specified compounds were generated within the filter. Further, the industry should develop alternative experimental approaches to address whether titanium dioxide particles embedded in cellulose acetate can affect the composition of MSS within the relevant temperature range. Moreover, the granulometry of filter titanium dioxide, and the procedure of its application on the filter are not provided and their effect on titanium dioxide particles emissions is not discussed.

Evaluation by the review panel: The only study that was performed was addressing particle transfer with an insufficient experimental set-up (only sham smoking with an unlit cigarette). Possible effects of titanium dioxide, for example due to function as a catalyst, on pyrolysis and smoke chemistry has not been addressed. According to the panel's opinion, it is not acceptable to omit the above-mentioned points, as a comprehensive report is requested by the Tobacco Product Directive (2014/40/EU art.6). Thus, the provided report does not comply with the requirements of Article 6 in the TPD. Further, in the industry report, titanium dioxide is only covered as an ingredient in cigarettes filters, but not in cigarette paper and tobacco. Test cigarettes contained 300 mg titanium dioxide per 600 cigarettes or 0.5 mg per cigarette filter. Products that contain titanium dioxide in other parts or in higher levels are not covered by the current industry report.

5.14.5 Toxicity and CMR properties

As specified in the Chapter 3 and 4, there are four main strategies for toxicological evaluation; these are assessed separately below, before a general conclusion is drawn.

A. Evaluation of **additive itself (ingestion)** – NOT ASSESSSED

As discussed in Chapter 4, evaluation of the toxicity of an additive due to ingestion has limited relevance for the evaluation of its toxicity when used as a tobacco additive. The industry report does not include an evaluation of the toxicity of titanium dioxide when ingested, and the physico-chemical characteristics of the titanium dioxide used in filter cigarettes (particularly the granulometry) as compared to the food additive are not described.

B. Evaluation of **additive itself (inhalation)** – PARTLY ASSESSED

The inhalation toxicity of titanium dioxide is poorly evaluated in the industry report, which states that Titanium dioxide is only applied in the cigarette filter material and therefore, should not be released in smoke and consequently, should not be of concern for inhalation. As mentioned above, only one literature study was cited in the report for the toxicological assessment of titanium dioxide as a tobacco additive. This study, Paschke *et al.* (9), was published by the industry and did not confirm any associations between occupational exposures to titanium dioxide and cancer risks. However, a recent independent publication questions this conclusion and suggests a positive relationship between titanium dioxide exposure and lung cancer mortality in titanium dioxide workers (10). The industry submission also refers to a report of NIOSH (National Institute for Occupational Safety and Health) that was released in 2011 (11), stating that the cited epidemiological studies do not provide clear evidence of elevated risks of lung cancer mortality or morbidity. The industry concluded from the NIOSH report and another publication (12) that titanium dioxide is not a carcinogen.

In 2015, French competent authority (Anses), submitted a proposal through the CLH process in order to classify titanium dioxide as carcinogen, category 1B, for the inhalation route. This proposal was evaluated by the committee for risk assessment (RAC) in 2017. As there are no robust carcinogenicity studies in species other than rats and as epidemiology studies did not consistently suggest an association between occupational exposure to titanium dioxide and lung cancer mortality, RAC proposed to classify titanium dioxide as carcinogen, category 2, for the inhalation route. Although some studies used in this opinion are also mentioned in the industry report, the RAC opinion itself is hardly quoted and discussed in the industry report. The European Commission has classified titanium dioxide as a "carcinogen category 2", the classification becomes effective on September 9th, 2021 (3). From that date, tobacco and related products containing titanium dioxide do not comply with Article 7 (6e) of the TPD. The industry acknowledged the pending classification of titanium dioxide as category 2 carcinogen, although no decision had been reached at the time of publishing their report.

C. Evaluation of the **pyrolysis product** – NOT ASSESSED

No pyrolysis products are expected. However, the probable function of titanium dioxide as catalyst on the gas phase reactions or its reaction with the MSS compounds during the pyrolysis phase are not evaluated. There is no evaluation in terms of oral nor inhalation toxicity for this part in the industry report either.

D. Evaluation of **mainstream smoke (comparative testing)** – NOT ASSESSED

The industry report did not include comparative testing or literature review for the evaluation of mainstream smoke. There is no evaluation for this part in the industry report. This is a major shortcoming of the industry's assessment of titanium dioxide.

Evaluation by the review panel: The industry report did not provide a sufficient assessment of the toxicity of titanium dioxide in the context of smoking. Nevertheless, the European Commission has classified titanium dioxide as a "carcinogen category 2", the classification becomes effective on

September 9th, 2021. From that date, tobacco and related products containing titanium dioxide do not comply with Article 7 (6e) of the TPD.

5.14.6 Addictiveness, Inhalation facilitation and Nicotine uptake

Concerns to be addressed:

Addictiveness & Inhalation facilitation: There is currently no data available on the effects of titanium dioxide on addictiveness in general or inhalation facilitation or nicotine uptake specifically.

Industry experiments: The industry report also did not provide any data on these topics, despite the requirement in the TPD (art. 6.2).

Evaluation of the review panel: The industry has not provided any assessment regarding effects on addictiveness, inhalation facilitation, and nicotine uptake by titanium dioxide as a tobacco additive. Even though the review panel has no knowledge of such effects, provision of such data is required by TPD (art. 6.2).

5.14.7 Characterizing flavor

Whether the additive titanium dioxide can be responsible for introducing a characterizing flavor or odor to tobacco products has not been addressed in the industry report. However, as titanium dioxide is not known to be a flavoring compound and not likely to transfer into smoke, no such effect is expected. However, the possible catalytic effects of titanium dioxide on the MSS components cannot exclude a reaction leading to secondary products having flavor characteristics and this mechanism is not assessed in the industry report.

5.14.8 Overall conclusion on additive

The industry submitted only a very limited evaluation on titanium dioxide as an additive in cigarettes. The assessment was exclusively performed for titanium dioxide application in the cigarette filter and only in the application level of 0.5 mg per cigarette. The only study that was performed was addressing particle transfer with an insufficient experimental set-up (only sham smoking with an unlit cigarette). Based on this experiment, transfer of titanium dioxide particles into mainstream smoke during smoking cannot be ruled out. Possible effects of titanium dioxide, for example due to function as a catalyst, on pyrolysis and smoke chemistry were not addressed. Assessment of addictiveness, inhalation facilitation and characterizing flavor, despite being required by the TPD, were not provided either.

Most importantly, the report on titanium dioxide hardly reviews data on toxicity of this additive. Instead, the decision by ECHA committee on risk assessment (RAC) to classify titanium dioxide as carcinogen, category 2, was questioned without thorough discussion of the literature. To avoid discussions of toxicity in the context of smoking, the report relies on the statement that no titanium dioxide is released from the filter, but fails to provide sufficient evidence for this statement. The European Commission has classified titanium dioxide as a “carcinogen category 2”, the classification becomes effective on September 9th, 2021 (3). It should be noted that the classification as a carcinogen by inhalation applies only to mixtures in powder form containing 1 % or more of titanium dioxide in the form of or incorporated in particles with aerodynamic diameter ≤ 10 µm. However, it cannot be concluded that there are no titanium dioxide particles in the smoke in that form without combustion experiments. Therefore, from that date, tobacco and related products containing titanium dioxide do not appear to comply with Article 7 (6e) of the TPD, unless proven differently.

Products that contain titanium dioxide in tobacco, or in any other component, including cigarette

paper, or in higher amounts than 0.5 mg are not covered by the submitted report and do not comply with Article 6 of directive 2014/40/EU.

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5.15 Brief statement on known risks and missing data on diacetyl

No report on diacetyl has been submitted by the industry consortium, as they state that this additive is not used in any of their cigarettes or roll your own (RYO) products. Thus, cigarettes and RYO products that contain diacetyl as an additive do not comply with the TPD, because the enhanced reporting obligations have not been fulfilled. The review panel performed an independent and non-comprehensive literature review to provide a brief statement on known risks and missing data on diacetyl.

5.15.1 Abstract

Diacetyl (CAS 431-03-8) is naturally occurring in various foods, especially in dairy products. In some types of tobacco, it is naturally occurring but might be added as a flavoring to the tobacco product as well in low amounts. In a previous study, 285 µg diacetyl per cigarette was measured in mainstream smoke using the ISO smoking regime. Diacetyl is a volatile compound, mainly produced by the pyrolysis of sugars, such as sucrose and glucose. The industry did not submit a report on diacetyl and therefore, the review panel was not able to evaluate their assessment of comparative testing, toxicity, addictiveness, inhalation facilitation, and characterizing flavor. In an independent literature review performed by the review panel, studies on workplace exposure and inhalation toxicity were retrieved, showing that diacetyl can cause mild to life-threatening airway obstruction. Furthermore, as a flavoring, diacetyl increases attractiveness of tobacco by creating or contributing to a certain flavor/taste.

5.15.2 Diacetyl in cigarette smoke

Diacetyl (CAS 431-03-8) is naturally occurring in various foods, especially dairy products. As a food flavoring agent it mostly gives a buttery taste to the product. It is “generally recognized as safe” (GRAS) by the Flavor and Extract Manufacturers Association of the United States (FEMA) for oral uptake. In some types of tobacco, it is naturally occurring but might be added as a flavoring to the tobacco product as well. However, the major amount of diacetyl in cigarette smoke is created during pyrolysis of constituents such as sugars, including sucrose and glucose (1-3). Pierce *et al.* found 285 µg diacetyl per cigarette in mainstream smoke when using the ISO smoking regime. Additionally, they calculated the concentration of diacetyl in ppm based on the puff volume that was taken and proposed an exposure of 250 ppm diacetyl per cigarette when using the ISO method (1). The national Institute for Occupational Safety and Health (NIOSH) recalculated the concentration in ppm given the fact that smokers dilute the concentrated mainstream smoke with surrounding air that contains far less diacetyl. They suggested an average exposure of 0.45 to 1.3 ppm per cigarette. The 8-hour time-weighted average equivalent of a smoker who smokes 20 cigarettes per day was 0.170 to 0.5 ppm, considering the time the smoker does not smoke (4). Fujioka *et al.* did not use a standardized smoking procedure and found 301 to 433 µg diacetyl per cigarette (5).

5.15.3 Toxicological concerns regarding occupational exposure

High occupational exposure to diacetyl was linked to development of severe bronchiolitis obliterans in food and flavoring workers (6). Bronchiolitis obliterans (BO) is a rare fibrotic lung disease with obstructions in the small airways. It has been described to occur after lung transplant or injury due to inhalation of toxicants like sulfur, mustard gas or nitrogen oxides (7). After development of BO by eight workers of a microwave-popcorn plant in Missouri, exposure to diacetyl was analyzed and correlated with lung function parameters of workers (8, 9). Higher exposures correlated with worse spirometry results. In the mixing room, the maximum exposure was determined as 98 ppm, the mean as 32 ppm (9). In another plant, peak exposures of over 80 ppm for several minutes were measured (10).

Studies on inhalation toxicology have been conducted in rodents. In one study, mice developed necrosis in the upper airways under subacute exposure to diacetyl (200 or 400 ppm, 6h/day for 5 days). Six of ten animals of the 200 ppm group were euthanized in moribund conditions and all animals in the 400 ppm group died or were euthanized in moribund conditions during the treatment period. Studies in rats showed after acute exposure for 6 hours necrosis of the bronchiolar epithelium (203 to 352 ppm) (11, 12). Single intratracheal instillation of diacetyl that avoids nasal scrubbing in the obligate nose-breathers induced BO in rats (13).

The link between diacetyl and generation of BO has been challenged in some publications, as reviewed by the Scientific Committee on Occupational Exposure Levels (SCOEL) in their recommendation on this substance. Nevertheless, SCOEL concluded that the evidence is sufficient that diacetyl can cause mild to life-threatening airway obstruction and suggested an 8-hour TWA-OEL (Time Weighted Average – Occupational Exposure Limit) of 0.02 ppm. A STEL (Short Term Exposure Limit), representing an exposure peak for 15 minutes, of 0.1 ppm was recommended (14). NIOSH recommended an 8-hour TWA-REL of 0.005 ppm and a STEL of 0.025 ppm (4).

5.15.4 Toxicological concerns regarding tobacco smoking

Cigarette smoking has not been shown to be a risk factor in generation of BO, neither in the occupational exposure settings nor due to diacetyl in mainstream smoke alone (1). Pierce *et al.* have reviewed the discussion about a "protective effect" of smoking and the explanation that ciliated cells are replaced by squamous epithelium in smokers (squamous metaplasia). However, the authors concluded that a "protective effect" of cigarette smoking against development of BO is not scientifically sound (1). NIOSH compared BO with chronic obstructive pulmonary disease (COPD). COPD is strongly associated with smoking and induces morphologic changes that are additional to the obstruction and fibrosis that are typical for BO as well. They hypothesized that diacetyl and related compounds might contribute to the development of obstructive lung diseases (4).

The Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) raised concerns regarding uncertainties about genotoxicity and unknown carcinogenicity of diacetyl (15, 16). Further, possible hazards of diacetyl are discussed in the context of its use in liquids for electronic cigarettes. Addition of diacetyl to e-liquids is prohibited in Germany (17).

5.15.5 What we do not know

It is still unclear how smokers are affected by diacetyl and related compounds in the mainstream smoke and to what extent it contributes to the hazard of smoking. One concern is the inhalation toxicity of the substance and the development of obstructive lung diseases. Furthermore, as a flavoring, diacetyl could increase attractiveness and addictiveness of tobacco by creating or rounding off a certain taste. It might be that precursor substances that pyrolyze into diacetyl contribute also to increase the amount of diacetyl in cigarette smoke. Given the fact that the main source of diacetyl in mainstream smoke stems from pyrolysis, regulation of diacetyl as an additive could be complemented by regulation of additives that promote diacetyl generation.

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Annex I: Outcomes table

Table AI.1 Overview of outcomes: General information about the 15 additives as provided in the industry report (grey columns: Low, Max and Max-plus application levels as targeted levels, a remark about volatility of the additive, transfer rates and pyrolysis products as provided in the industry reports); Industry's conclusion regarding influence of the 15 additives on smoke chemistry, toxicity, addictiveness, inhalation facilitation, and characterizing flavor (red columns); Review panel's assessment of the additives' influences on smoke chemistry, toxicity, addictiveness, inhalation facilitation, and characterizing flavor on the basis of provided data and independently performed literature searches (blue columns). For independent assessment of the industry's chemical analysis, the criteria described in Chapter 3 were applied. The addressed concerns in this table are only exemplary. More details are given in the individual additive reports. Furthermore, some limitations and shortcomings of the industry's approach are briefly summarized under "General remarks". A more extensive discussion on this topic is given in Chapter 4.

	As provided in industry reports				Industry conclusion					Review panel's assessment of provided (and independent) data					
Additive	Tested applicati¬on level	Volatile?	Transfer rates	Main pyrolysis products	Chemical analysis: overall effect	Toxicity	Addictive-ne¬ss	Inhalation facilitation	Characterizing flavor	Chemical analysis, re-eval.: carbonyl comp.	Chemical analysis, re-eval.: other comp.	Toxicity	Addictiveness	Inhalation facilitation	Characterizing flavor
Review panel's general remarks (see Chapter 4)			Only assessed for: cocoa, geraniol, glycerol, guaiacol, licorice, maltol, menthol, propylene glycol, TiO2	No new experiments were performed	Limitations in methodology (e.g., statistical analysis by industry was likely to cause false negative results)	Limitations in method-ology (e.g., study design was not sufficient to evaluate CMR prop.)	Limitations in method-ology (e.g., important Endpoints on dependence potential were not assessed)	Limitations in method-ology (e.g., only descript. statistics were provided for smoking behavior param.)	Only assessed for: Carob bean, cocoa, fenugreek, fig, geraniol, guaiacol, licorice, menthol. Limitations in methodology	Review panel considers industry assessment as insufficient (see Chapter 4). Thus, evidence from independent literature was used when available, but no comprehensive literature review was performed. The review panel performed an evaluation of hazard classification of pyrolysis products. For MSS chemical analysis, usefulness of data provided by industry was limited (e.g., only ISO smoking regime was used, pyrolysis products were not included in analyte list, high standard deviations for some experiments), thus a re-analysis of the chemical data was performed by the review panel. For the remaining endpoints, addressed concerns are exemplary. Further details are given in the individual additive reports.					
Carob bean	Low 0.2% Max 0.4% Max-plus 0.6%	Carob bean is a complex mixture of mainly non-volatile compounds	Unknown but unlikely	Acetic acid (24.5%), acetol (3.4%), furfural (1.5%), pyruvaldehyde (1%); diluent propylene glycol was found at 65.4%	No statistically significant overall effect	No effect	No effect	No effect	No effect	No increase of carbonyl emissions at tested levels	No additive-level related MSS effects in industry study. (Effects on MSS described in literature).	Pyrolysis product furfural has CMR properties, not followed up in MSS analysis.	Potential MAO inhibition has not been addressed.	Potential alteration of smoke pH has not been addressed.	Impact on tobacco flavor has likely been underestimated
Cocoa	Low 0.5% Max 1.0% Max-plus 1.5%	Cocoa is a mixture, some components are volatile	Varies	Acetic acid (27.2%), acetol (6.6%), furfuryl alcohol (6.6%), caffeine (4.0%), pyrrole (2.8%), furfural + cyclopentanone (2.1%), phenol (1.6%) cresol + pyridenediol (1.4%), 2-butanone (0.9%), toluene (0.7%), styrene (0.2%)	No statistically significant overall effect	No effect	No effect	No effect	No effect	No increase of carbonyl emissions at tested levels	Additive level-related increase of cadmium	Pyrolysis product furfural has CMR properties, not followed up in MSS analysis.	Potential MAO inhibition has not been addressed.	Potential alteration of smoke pH has not been addressed.	Impact on tobacco flavor has likely been underestimated

Fenugreek	Low 0.01% Max 0.02% Max-plus 0.03%	Fenugreek extract is a complex mixture, main components are not volatile	Unknown, but unlikely for main constituents	Ethyl linoleate (37.4%), ethyl palmitate (14.8%), ethyl stearate (10.6%), palmitic acid (6%), hydroxydimethylfuranone (3.4%)	No statistically significant overall effect	No effect	No effect	No effect	No effect	No increase of carbonyl emissions at tested levels	No additive-level related MSS effects in industry study.	Pyrolysis products (furfural, benzene, toluene, 2-butenal) have CMR properties, some were not followed up in MSS analysis.	Potential MAO inhibition (due to combustion of sugars) has not been addressed. However, application level is very low.	Potential alteration of smoke pH has not been addressed. However, application level is very low.	Impact on tobacco flavor has likely been underestimated
Fig	Low 0.025% Max 0.15% Max-plus 0.30%	Fig juice concentrate is a complex mixture, main components are not volatile	Unknown, but unlikely for main constituents	Acetic acid (45.1%), furfural (24.5%), sorbic acid (10.2%), butanediol (3.7%); unknown emission (8.6%)	No statistically significant overall effect	No effect	No effect	No effect	No effect	No increase of carbonyl emissions at tested levels	No additive-level related MSS effects in industry study.	Pyrolysis product furfural has CMR properties, not followed up in MSS analysis.	Potential MAO inhibition (due to combustion of sugars) has not been addressed.	Potential alteration of smoke pH has not been addressed.	Impact on tobacco flavor has likely been underestimated
Geraniol	Geraniol Low 0.015% Max 0.030% Max-plus 0.045%	Yes	7-8 %	Citral (4.6%), beta-myrcene (3%), ocimene (1.8%), neryl acetate (1.3%), alloocimene (0.7%), menthatriene (0.5%), limonene (0.4%)	No statistically significant overall effect	No effect	No effect	No effect	No effect	Low quality of data does not allow conclusion.	Increase of nitrogen oxides, but quality of data low.	Increase in toxicity is unlikely (given the low application level), but cannot be excluded.	Not adequately assessed: only as part of a mixture	Not adequately assessed: only as part of a mixture. Activation of the cooling receptor TRPM8 was not addressed.	Impact on tobacco flavor has likely been underestimated
Glycerol	Low 2.5% Max 5.0% Max-plus 6%	No	4.5 % (in the literature up to 8%)	Glycerol (99.8%)	Decrease of benzo[a]pyrene, NAB, catechol, hydroquinone, m+p cresol, o cresol, phenol, quinoline. Increase of glycerol.	No effect	No effect	No effect	Not assessed	No increase of carbonyl emissions at tested levels.	Additive level-related increase of ammonia and water.	Industry's assessment insufficient.	Industry's assessment insufficient. However, no previously identified concerns regarding addictiveness.	Inhalation facilitation due to humidification not addressed.	No such effect expected
Guaiacol	Low 0.0005% Max 0.001% Max-plus 0.0015%	Yes	unknown	Guaiacol (92.5%), guaiacol acetate (6.3%), indanone (0.7%), dimethoxybenzene (0.3%), chinnoline (0.2%)	No statistically significant overall effect	No effect	No effect	No effect	No effect	Low quality of data does not allow conclusion.	No additive-level related MSS effects in industry study. (MSS effects described in literature.)	Irritative effects have not been addressed. However, application level is very low.	Industry's assessment insufficient. However, no previously identified concerns regarding addictiveness.	Anesthetic effects are not assessed.	Impact on tobacco flavor has likely been underestimated
Guar gum	Low 0.5% Max 1.0% Max-plus 1.5%	No	Not applicable	Hydroxymethylfurfural (13.4%), acetol (11.9%), acetic acid (9.9%), methyl pyruvate (6.1%), furfural (6.0%), cresol (0.9%), benzene (0.7%), 2-butanone (0.7%), toluene (0.5%), 2-butenal (0.2%)	Increase of formaldehyde and cadmium.	No effect	No effect	No effect	Not assessed	Almost all carbo¬nyls increase with guar gum concen-trations. The increase in formaldehyde is seen as significant and relevant.	Substantial variations of water and nitrogen oxides have not been explained by industry. (MSS effects described in literature).	Pyrolysis products (furfural, benzene, toluene, 2-butenal) have CMR properties, some were not followed up in MSS analysis.	Not adequately assessed: only as part of a mixture. MAO inhibitors (aldehydes) were increased in MSS.	Not adequately assessed: only as part of a mixture. Potential alteration of smoke pH has not been addressed.	Influence of guar gum and its pyrolysis products on odor and taste was not assessed.

Licorice	Low 0.6% Max 1.2% Max-plus 1.8%	Licorice is a mixture, most components are not volatile	Not applicable	Acetic acid (42%), acetol (11.9%), furfuryl alcohol (11.7%), diacetyl (4.1%), acetol acetate (2.0%), phenol (1.4%), cresol (0.2%), pyridine/pyrrole (0.2%), furfural (0.2%)	No statistically significant overall effect	No effect	No effect	No effect	No effect	No increase of carbonyl emissions at tested levels.	Increase of cadmium. (MSS effects described in literature).	Pyrolysis products (furfuryl alcohol, phenol, furfural, diacetyl) have CMR properties or cause obstructive lung injury, some were not followed up in MSS analysis	Potential MAO inhibition (due to combustion of sugars) has not been addressed.	Potential alteration of smoke pH has not been addressed.	Impact on tobacco flavor has likely been underestimated
Maltol	Low 0.005% Max 0.01% Max-plus 0.015%	Not very volatile in ambient conditions	4.3 – 5.2 %	Acetoxymethyl pyranone (0.2%); maltol transfers mostly intact	No statistically significant overall effect	No effect	No effect	No effect	Not assessed	Low quality of data does not allow conclusion.	Additive level-related increase of nitrogen oxides.	Increase in toxicity is unlikely (given the low application level), but cannot be excluded.	Not adequately assessed: only as part of a mixture. GABAA receptor inhibition has not been addressed.	Not adequately assessed: only as part of a mixture	Influence of maltol on odor and taste was not assessed.
Menthol	Low 0.1% Max 1.2% Max-plus 1.8%	Yes	9.1 – 11.1 % (in the literature up to 30%)	Menthone (0.9%), menthene (0.1%); menthol transfers mostly intact	Increase of menthol	No effect	No effect	No effect	Characterizing flavor at 1.2% or higher application	No increase of carbonyl emissions at tested levels	Increase of NAT. (MSS effects described in literature).	Indirect effects on toxicity (e.g., due to increased puff volume) have not been addressed.	Effects of menthol on addictiveness (e.g., alteration of nicotine levels, masking of aversive sensory experiences, serving as conditioned cue) have not been addressed.	Menthol's ability to facilitate inhalation via activation of the cooling receptor TRPM8 was not addressed.	Contrary to already existing literature. Effect on palatability and attractiveness due to cooling effect has not been addressed
Propylene Glycol	Low 2.5% Max 5.0% Max-plus 6%	No	Below 1 % (in the literature 7.3 – 8.8 %)	1,3-Propylene glycol (6.2%), acetol or acetic anhydride (4.7%), pyruvaldehyde (2.8%); propylene glycol transfers mostly intact	Decrease of m+p cresol and phenol. Increase of propylene glycol.	No effect	No effect	No effect	Not assessed	Low quality of data does not allow conclusion.	Increase of cadmium and additive level-related increase of nitrogen oxides.	Pyrolysis product pyruvaldehyde has CMR properties, not followed up in MSS analysis.	Industry's assessment insufficient. However, no previously identified concerns regarding addictiveness.	Inhalation facilitation due to humidification not addressed.	No such effect expected
Sorbitol	Low 0.6% Max 1.2% Max-plus 1.8%	No	Not assessed	Furfural (31.4%), propylfuran (9.7%), acetylfuran (7.7%), furanone (6.4%), methoxycyclopentenone (5.2%)	Increase of acrolein and formaldehyde.	No effect	No effect	No effect	Not assessed	Relevant increase in carbonyl formation (esp. acrolein and formaldehyde) is attributed to additive	Additive-level related increase of cadmium	Increase of toxic carbonyls. Pyrolysis product furfural has CMR properties, not followed up in MSS analysis.	MAO inhibitors (aldehydes) were increased in MSS.	Inhalation facilitation due to humidification not addressed.	Influence of sorbitol and its pyrolysis products on odor and taste was not assessed.
Titanium dioxide	0.5 mg per cigarette filter (not tested)	No	Not assessed	Not applicable	No comparative testing	Not assessed	Not assessed	Not assessed	Not assessed	No comparative testing	Insufficient evaluation of transfer of titanium dioxide to smoke	EU Carc. 2 classification of titanium dioxide	Not assessed	Not assessed	Not assessed
Diacetyl	No report provided									The inhalation toxicity of diacetyl and the development of obstructive lung diseases after exposure to it are concerns. Furthermore, as a flavoring, diacetyl could increase attractiveness of tobacco.					

Annex II: Carbonyl emissions

Table AII.1 Overview of carbonyl emissions: Mean values of carbonyl emissions derived from the test cigarettes containing the single additives and from the corresponding additive-free reference cigarettes were extracted from the industry reports and ratios $\left(\frac{\text{Test cigarette}}{\text{Reference cigarette}}\%\right)$ were calculated. Data provided by the industry for geraniol, guaiacol, maltol, and propylene glycol were of too low quality to allow conclusions. Although data for sorbitol and guar gum were of low quality as well, the increases of some carbonyl emissions were still apparent and considered relevant and significant by the review panel and acknowledged as significant by the industry ("increase" as "overall effect").

Additive	Industry data and interpretation					Interpretation from reviewers
	Compound	Overall effect	Comparison to reference (ratio)			
			Low	Max	*Max-plus*	
Carob bean	Acetaldehyde	none	98%	99%	90%	No increase of carbonyl emissions at tested levels
	Acetone	none	101%	101%	93%	
	Acrolein	none	94%	90%	83%	
	Butyraldehyde	none	97%	101%	93%	
	Crotonaldehyde	none	103%	102%	91%	
	Formaldehyde	none	97%	89%	87%	
	Propionaldehyde	none	100%	101%	92%	
Cocoa	Acetaldehyde	none	93%	104%	93%	No increase of carbonyl emissions at tested levels
	Acetone	none	95%	104%	96%	
	Acrolein	none	88%	98%	89%	
	Butyraldehyde	none	93%	105%	94%	
	Crotonaldehyde	none	95%	103%	99%	
	Formaldehyde	none	89%	97%	90%	
	Propionaldehyde	none	94%	103%	93%	
Diacetyl	Not provided					
Fenugreek	Acetaldehyde	none	98%	94%	98%	No increase of carbonyl emissions at tested levels
	Acetone	none	100%	94%	102%	
	Acrolein	none	90%	86%	88%	
	Butyraldehyde	none	101%	95%	99%	
	Crotonaldehyde	none	95%	94%	97%	
	Formaldehyde	none	79%	82%	87%	
	Propionaldehyde	none	102%	96%	101%	
Fig	Acetaldehyde	none	95%	101%	100%	No increase of carbonyl emissions at tested levels
	Acetone	none	94%	102%	102%	
	Acrolein	none	90%	95%	94%	
	Butyraldehyde	none	96%	100%	102%	
	Crotonaldehyde	none	92%	104%	105%	
	Formaldehyde	none	86%	92%	92%	
	Propionaldehyde	none	96%	103%	101%	
Geraniol	Acetaldehyde	none	149%	123%	119%	Low quality of data does not allow conclusion.
	Acetone	none	144%	121%	114%	
	Acrolein	none	151%	123%	124%	
	Butyraldehyde	none	143%	124%	115%	
	Crotonaldehyde	none	149%	138%	108%	
	Formaldehyde	none	159%	134%	130%	
	Propionaldehyde	none	147%	127%	119%	
Glycerol	Acetaldehyde	none	96%	100%	93%	No increase of carbonyl emissions at tested levels
	Acetone	none	98%	96%	89%	
	Acrolein	none	99%	110%	108%	
	Butyraldehyde	none	92%	94%	87%	

	Crotonaldehyde	none	94%	95%	76%	
	Formaldehyde	none	91%	100%	85%	
	Propionaldehyde	none	97%	103%	95%	
Guaiacol	Acetaldehyde	none	149%	128%	131%	Low quality of data does not allow conclusion.
	Acetone	none	142%	125%	128%	
	Acrolein	none	154%	137%	127%	
	Butyraldehyde	none	141%	123%	129%	
	Crotonaldehyde	none	162%	130%	125%	
	Formaldehyde	none	155%	139%	131%	
	Propionaldehyde	none	150%	130%	129%	
Guar gum	Acetaldehyde	none	127%	125%	154%	Almost all carbo-nyls increase with guar gum concen-trations. The increase in formaldehyde is seen as significant and relevant.
	Acetone	none	125%	121%	148%	
	Acrolein	none	130%	135%	163%	
	Butyraldehyde	none	125%	120%	147%	
	Crotonaldehyde	none	132%	131%	169%	
	Formaldehyde	**increase**	**130%**	**168%**	**200%**	
	Propionaldehyde	none	129%	126%	147%	
Licorice	Acetaldehyde	none	106%	102%	98%	No increase of carbonyl emissions at tested levels
	Acetone	none	107%	103%	97%	
	Acrolein	none	105%	96%	96%	
	Butyraldehyde	none	104%	103%	96%	
	Crotonaldehyde	none	115%	102%	97%	
	Formaldehyde	none	114%	102%	114%	
	Propionaldehyde	none	106%	102%	96%	
Maltol	Acetaldehyde	none	136%	133%	117%	Low quality of data does not allow conclusion.
	Acetone	none	131%	127%	115%	
	Acrolein	none	144%	141%	117%	
	Butyraldehyde	none	131%	138%	115%	
	Crotonaldehyde	none	131%	138%	115%	
	Formaldehyde	none	147%	153%	124%	
	Propionaldehyde	none	135%	135%	117%	
Menthol	Acetaldehyde	none	99%	95%	94%	No increase of carbonyl emissions at tested levels
	Acetone	none	101%	98%	98%	
	Acrolein	none	89%	90%	85%	
	Butyraldehyde	none	101%	96%	86%	
	Crotonaldehyde	none	101%	96%	86%	
	Formaldehyde	none	99%	92%	91%	
	Propionaldehyde	none	99%	96%	94%	
Propylene Glycol	Acetaldehyde	none	130%	119%	136%	Low quality of data does not allow conclusion.
	Acetone	none	127%	114%	129%	
	Acrolein	none	132%	126%	140%	
	Butyraldehyde	none	125%	110%	125%	
	Crotonaldehyde	none	133%	111%	129%	
	Formaldehyde	none	127%	129%	146%	
	Propionaldehyde	none	135%	129%	149%	
Sorbitol	Acetaldehyde	none	112%	115%	147%	Relevant increase in carbonyl formation (esp. formaldehyde) is attributed to additive.
	Acetone	none	111%	111%	139%	
	Acrolein	**increase**	**115%**	**128%**	**181%**	
	Butyraldehyde	none	110%	108%	134%	
	Crotonaldehyde	none	94%	113%	170%	
	Formaldehyde	**increase**	**108%**	**125%**	**202%**	
	Propionaldehyde	none	113%	113%	148%	
Titanium dioxide	Not provided					

Annex III: Pyrolysis product table

Table AIII.1: overview of pyrolysis products: Pyrolysis products of each additive are listed with their CAS numbers, relevant hazard classifications from Table 3, CLP Regulation (legally binding) or from ECHA Classification & Labelling (C&L) inventory (not legally binding), and their IARC classifications (if applicable). CMR properties are highlighted in bold. Further details are given at the end of the table.

Additive	Max. application level	Pyrolysis product name	Pyrolysis product %	CAS No.	Legally binding?	Classification, Hazard class	IARC classification, year
Carob bean	0.6%	Acetic acid	24.5%	64-19-7	yes	Skin Corr. 1A	
Carob bean	0.6%	Acetol	3.4%	116-09-6			
Carob bean	0.6%	Furfural	1.5%	98-01-1	yes	Carc. 2; Eye Irrit. 2; STOT SE 3; Skin Irrit. 2	3, 1995
Carob bean	0.6%	Pyruvaldehyde	1.0%	78-98-8	no	Skin Sens.1B; Eye Dam. 1; Muta. 2	3, 1991
Cocoa	1.5%	Acetic acid	27.2%	64-19-7	yes	Skin Corr. 1A	
Cocoa	1.5%	Acetol	6.6%	116-09-6			
Cocoa	1.5%	Furfuryl alcohol	6.6%	98-00-0	yes	Carc. 2; STOT RE 2 *; Eye Irrit. 2; STOT SE 3	2B, 2019
Cocoa	1.5%	Caffeine	4.0%	58-08-2			3, 1991
Cocoa	1.5%	Pyrrole	2.8%	109-97-7			
Cocoa	1.5%	Furfural *	2.1%	98-01-1	yes	Carc. 2; Eye Irrit. 2; STOT SE 3; Skin Irrit. 2	3, 1995
Cocoa	1.5%	Cyclopentanone *	2.1%	120-92-3	yes	Eye Irrit. 2; Skin Irrit. 2	
Cocoa	1.5%	Phenol	1.6%	108-95-2	yes	Muta. 2; STOT RE 2 *; Skin Corr. 1B	3, 1999
Cocoa	1.5%	Cresol **	1.4%	108-39-4 [m] 95-48-7 [o] 106-44-5 [p] 1319-77-3 [mix]	yes	Skin Corr. 1B	
Cocoa	1.5%	Pyridinediol **	1.4%	3543-02-0 [3,5-P] 16867-04-2 [2,3-P] 84719-31-3 [2,4-P] 626-06-2 [2,6-P] 10182-48-6 [3,4-P]	no	CAS nr 16867-04-2: Skin Irrit. 2; Eye Irrit. 2; STOT SE 3	
Cocoa	1.5%	2-butanone	0.9%	78-93-3	yes	Eye Irrit. 2; STOT SE 3	
Cocoa	1.5%	toluene	0.7%	108-88-3	yes	Skin Irrit 2; STOT SE 3; STOT RE 2; Repr 2	3, 1999
Cocoa	1.5%	styrene	0.2%	100-42-5	yes	Skin Irrit 2; Eye Irrit 2; STOT RE 1; Repr 2	2A, 2019
Fenugreek extr.	0.03%	Ethyl linoleate	37.4%	544-35-4	no	Skin Irrit. 2	

Fenugreek extr.	0.03%	Ethyl palmitate	14.8%	628-97-7			
Fenugreek extr.	0.03%	Ethyl stearate	10.6%	111-61-5	no	Skin Irrit. 2; Eye Irrit. 2; STOT SE 3	
Fenugreek extr.	0.03%	Palmitic acid	6.0%	57-10-3	no	Skin Irrit. 2; Eye Irrit. 2; STOT SE 3	
Fenugreek extr.	0.03%	Hydroxydimethylfuranone	3.4%	28664-35-9			
Fenugreek extr.	0.03%	pyridine	0.4%	110-86-1			2B, 2019
Fenugreek extr.	0.03%	2-butanone	0.3%	78-93-3	yes	Eye Irrit. 2; STOT SE 3	
Fenugreek extr.	0.03%	benzene *	0.2%	71-43-2	yes	Skin Irrit 2; Eye Irrit 2; Muta 1B; Carc 1A; STOT RE 1	1, 2018
Fenugreek extr.	0.03%	methylbutenal *	0.2%	107-86-8	no	Skin Corr 1C; Skin Sens 1; Eye Dam 1	
Fenugreek extr.	0.03%	toluene **	0.2%	108-88-3	yes	Skin Irrit 2; STOT SE 3; STOT RE 2; Repr 2	3, 1999
Fenugreek extr.	0.03%	pentanol **	0.2%	71-41-0	yes	Skin Irrit 2; STOT SE 3	
Fenugreek extr.	0.03%	2-Butenal	0.1%	4170-30-3	yes	Skin Irrit 2; Eye Dam 1; STOT SE 3; Muta 2; STOT RE 2	3, 1995
Fenugreek extr.	0.03%	furfural	0.1%	98-01-1	yes	Carc 2; Eye Irrit 2; STOT SE 3; Skin Irrit 2	3, 1995
Fenugreek oil	0.03%	diethyl tartrate	74.3%	87-91-2			
Fenugreek oil	0.03%	acetic anhydride	4.7%	108-24-7	yes	Skin Corr. 1B	
Fenugreek oil	0.03%	Acetic acid	4.7%	64-19-7	yes	Skin Corr. 1A	
Fenugreek oil	0.03%	ethyl oleate	3.9%	111-62-6			
Fenugreek oil	0.03%	ethyl linoleate	2.4%	544-35-4	no	Skin Irrit. 2	
Fenugreek tinct.	0.03%	heptanoic acid	71.4%	111-14-8	yes	Skin Corr 1B	
Fenugreek tinct.	0.03%	Acetic acid	5.2%	64-19-7	yes	Skin Corr. 1A	
Fenugreek tinct.	0.03%	pyridine	3.4%	110-86-1			2B, 2019
Fenugreek tinct.	0.03%	vinylphenol	2.5%	2628-17-3	no	Skin Corr 1B; Skin Sens 1; Resp Sens 1	
Fenugreek tinct.	0.03%	Phenol	2.3%	108-95-2	yes	Muta. 2; STOT RE 2 *; Skin Corr. 1B	3, 1999
Fenugreek tinct.	0.03%	Furfural	0.5%	98-01-1	yes	Carc 2; Eye Irrit 2; STOT SE 3; Skin Irrit 2	3, 1995
Fig	0.30%	Acetic acid	45.1%	64-19-7	yes	Skin Corr. 1A	
Fig	0.30%	Furfural	24.5%	98-01-1	yes	Carc. 2; Eye Irrit. 2; STOT SE 3; Skin Irrit. 2	3, 1995

Fig	0.30%	Sorbic acid	10.2%	110-44-1	no	Skin Irrit. 2; Eye Irrit. 2; STOT SE 3	
Fig	0.30%	Butanediol	3.7%	584-03-2 [1,2-B.] 107-88-0 [1,3-B.] 110-63-4 [1,4-B.] 513-85-9 [2,4-B.]		1,4-Butanediol:	
Geraniol	0.05%	Citral	4.6%	5392-40-5	yes	Skin Irrit. 2; Skin Sens. 1	
Geraniol	0.05%	Betamyrcene	3.0%	123-35-3	no	Skin Irrit. 2;	2B, 2019
Geraniol	0.05%	Ocimene	1.8%	13877-91-3	no	Skin Irrit. 2; Eye Irrit 2;	
Geraniol	0.05%	Neryl acetate	1.3%	141-12-8	no	Skin Irrit. 2; Skin Sens. 1B;	
Geraniol	0.05%	Alloocimene	0.7%	673-84-7	no	Skin Irrit. 2; Skin Sens. 1; Eye Irrit 2;	
Geraniol	0.05%	Menthatriene	0.5%	18368-95-1		no results on June, 25th, July, 28th	
Geraniol	0.05%	Limonene	0.4%	138-86-3	yes	Skin Irrit. 2; Skin Sens. 1;	
Glycerol	6.0%	Glycerol	99.8%	56-81-5	no	Not classified	
Guaiacol	0.0015%	Guaiacol	92.5%	90-05-1	yes	Eye Irrit. 2; Skin Irrit. 2	
Guaiacol	0.0015%	Guaiacol acetate	6.3%	613-70-7	no	Skin Irrit. 2	
Guaiacol	0.0015%	Indanone	0.7%	83-33-0 [-1-one] 615-13-4 [-2-one]			
Guaiacol	0.0015%	Dimethoxybenzene	0.3%	91-16-7 [1,2-] 151-10-0 [1,3-] 150-78-7 [1,4-]			
Guaiacol	0.0015%	Cinnoline	0.2%	253-66-7			
Guar gum	1.50%	Hydroxymethylfurfural	13.4%	67-47-0	no	Skin Irrit. 2; Eye Irrit. 2; STOT SE 3;	
Guar gum	1.50%	Acetol	11.9%	116-09-6			
Guar gum	1.50%	Acetic acid	9.9%	64-19-7	yes	Skin Corr. 1A	
Guar gum	1.50%	Methyl pyruvate	6.1%	600-22-6	no	Skin Sens.1B; Eye Dam. 1; STOT SE 3	
Guar gum	1.50%	Furfural	6.0%	98-01-1	yes	Carc. 2; Eye Irrit. 2; STOT SE 3; Skin Irrit. 2	3, 1995
Guar gum	1.50%	Cresol	0.9%	108-39-4 [m] 95-48-7 [o] 106-44-5 [p] 1319-77-3 [mix]	yes	Skin Corr 1B	
Guar gum	1.50%	Benzene	0.7%	71-43-2	yes	Skin Irrit 2; Eye Irrit 2; Muta 1B; Carc 1A; STOT RE 1	1, 2018
Guar gum	1.50%	2-butanone	0.7%	78-93-3	yes	Eye Irrit 2; STOT SE 3	
Guar gum	1.50%	Toluene	0.5%	108-88-3	yes	Skin Irrit 2; STOT SE 3; STOT RE 2; Repr 2	3, 1999

Guar gum	1.50%	2-Butenal	0.2%	4170-30-3	yes	Skin Irrit 2; Eye Dam 1; STOT SE 3; Muta 2; STOT RE 2	3, 1995
Licorice	1.80%	Acetic acid	42.0%	64-19-7	yes	Skin Corr. 1A	
Licorice	1.80%	Acetol	11.9%	116-09-6			
Licorice	1.80%	Furfuryl alcohol	11.7%	98-00-0	yes	Carc. 2; STOT RE 2 *; Eye Irrit. 2; STOT SE 3	2B, 2019
Licorice	1.80%	Diacetyl	4.1%	431-03-8	no	Skin Irrit. 2; Skin Sens. 1; Eye Dam. 1; STOT RE 2 (Resp.)	
Licorice	1.80%	Acetol acetate	2.0%	592-20-1			
Licorice	1.80%	Phenol	1.4%	108-95-2	yes	Muta. 2; STOT RE 2 *; Skin Corr. 1B	3, 1999
Licorice	1.80%	Cresol	0.2%	108-39-4 [m] 95-48-7 [o] 106-44-5 [p] 1319-77-3 [mix]	yes	Skin Corr. 1B	
Licorice	1.80%	Pyridine *	0.2%	110-86-1			2B, 2019
Licorice	1.80%	Pyrrole *	0.2%	109-97-7			
Licorice	1.80%	Furfural	0.2%	98-01-1	yes	Carc. 2; Eye Irrit. 2; STOT SE 3; Skin Irrit. 2	3, 1995
Maltol	0.015%	Acetoxymethyl pyranone	0.2%	no CAS-No for that name			
Maltol	0.015%	Maltol	mostly intact	118-71-8			
Menthol	1.80%	Menthol	mostly intact	2216-51-5 (L) 1490-04-6 (rac.)	no	Skin Irrit. 2; Eye Irrit. 2	
Menthol	1.80%	Menthone	0.9%	14073-97-3 (L) 10458-14-7 (rac)	no	Skin Irrit. 2; Skin Sens. 1B	
Menthol	1.80%	Menthene	0.1%	5502-88-5			
Propylene glycol	6.00%	1,3-Propylene glycol	6.2%	504-63-2	no	Skin Irrit. 2	
Propylene glycol	6.00%	Acetol *	4.7%	116-09-6			
Propylene glycol	6.00%	Acetic anhydride *	4.7%	108-24-7	yes	Skin Corr. 1B	
Propylene glycol	6.00%	Pyruvaldehyde	2.8%	78-98-8	no	Skin Sens.1B; Eye Dam. 1; Muta. 2	3, 1991
Propylene glycol	6.00%	Propylene glycol	mostly intact	57-55-6			
Sorbitol	1.80%	Furfural	31.4%	98-01-1	yes	Carc. 2; Eye Irrit. 2; STOT SE 3; Skin Irrit. 2	3, 1995
Sorbitol	1.80%	Propylfuran	9.7%	4229-91-8			
Sorbitol	1.80%	Acetylfuran	7.7%	1192-62-7	no	Eye Irrit. 2	
Sorbitol	1.80%	Furanone	6.4%	497-23-4			
Sorbitol	1.80%	Methoxycyclopentenone	5.2%	22323-97-3	no	Skin Irrit. 2; Eye Irrit. 2; STOT SE 3	

Sorbitol	1.80%	Pyruvaldehyde	3.7%	78-98-8	no	Skin Sens.1B; Eye Dam. 1; Muta. 2	3, 1991
Sorbitol	1.80%	Dimethylformylfuran	3.4%	no CAS-No for that name			
Sorbitol	1.80%	Methylfuran	3.1%	534-22-5	no	Eye Irrit 2	
Sorbitol	1.80%	Methylfurfural	2.6%	620-02-0			
Sorbitol	1.80%	Methyl furanone	2.4%	1333-38-6			
Sorbitol	1.80%	Cyclopemenediol	2.4%	no CAS-No for that name			
Sorbitol	1.80%	benzenediol	2.3%	12385-08-9			
Sorbitol	1.80%	Furfuryl alcohol	2.3%	98-00-0	yes	Carc. 2; STOT RE 2 *; Eye Irrit. 2; STOT SE 3	2B, 2019
Sorbitol	1.80%	Butanediol	2.0%	584-03-2 [1,2-B.] 107-88-0 [1,3-B.] 110-63-4 [1,4-B.] 513-85-9 [2,4-B.]		1,4-Butanediol:	
Sorbitol	1.80%	Acetol	1.9%	116-09-6			
Sorbitol	1.80%	Cyclopentenedione	1.6%	930-60-9	no	Skin Sens 1	negative

Explanation on fenugreek: Three forms of fenugreek have been investigated in pyrolysis experiments. The maximum application level of all fenugreek forms was 0.03%.

Explanation of Legally binding and IARC:

Legally binding?	
yes	Entry copied from Table 3, CLP Regulation
no	Notifications from ECHA C&L Inventory https://echa.europa.eu/information-on-chemicals/cl-inventory-database

Not all entries have been considered, only when at least ~30% of notifiers have used that statement. All CMR classifications have been included

International Agency for Research on Cancer (IARC) evaluates agents with respect to their carcinogenic potential. These evaluations are not legally binding within the EU. The two classifications mentioned in the table are:

2B: Possibly carcinogenic to humans

3: Not classifiable as carcinogenic to humans

Explanation of asterisks:

* The pyrolysis experiment could not distinguish between both substances

** The pyrolysis experiment could not distinguish between both substances

Annex IV: Industry addendum

In June 2018, the Priority Additives Tobacco Consortium filed reports of 15 priority additives in the European Common Entry Gate (EU-CEG). The JATC WP 9 independent scientific review panel had an extensive and challenging task in reviewing these industry reports which led to many critical discussions. Additionally, the panel formulated a letter (June 2019) with initial observations in which the industry consortium was asked for a clarification and remarks concerning the priority additive reports. In September 2019, a response was received from the Priority Additives Tobacco Consortium.

In this addendum to the deliverable of WP 9, we would like to address the response and complementary information received from the Priority Additives Tobacco Consortium.

In some cases, extra or new information was provided by the Priority Additives Tobacco Consortium, which is respectfully acknowledged by the review panel. In other cases, an explanation or clarification was provided for why the requested information could not be delivered. The JATC WP 9 review panel does not accede to the reasoning that a literature search was thorough and unbiased just because it was executed by independent research companies, or that a method or finding from a literature study should be taken at face value because it was published in a peer-reviewed journal, as several of these studies include a conflict of interest statement because they were performed or funded by the tobacco industry. Overall, the provided information and explanation in the letter from the Priority Additives Tobacco Consortium has not changed the outlook of the independent review panel or the outcomes of their review of the industry reports.

The JATC WP 9 acknowledge the provided clarification of general comments and adaptations in the format.

- On our request for unsecure files, the Industry Consortium replied that unprotected files without password were provided. We understand that the files were *unprotected* (i.e. not having a password) but our question was : "we would invite you to provide *unsecure* files" , meaning files that allow copy-pasting text fragments to facilitate the reviewing process (such as looking up literature references). It seems as if this problem only occurred for the cocoa file, so this is of overall minor concern.
- We acknowledge the clarification of the use of SCHEER's advised format, and respect the note for future submission. Furthermore, the information of additives that are natural compounds formatted in line with Substance Identity Profiles is appreciated.

The JATC WP 9 acknowledges the provided complementary information and clarification by the Priority Additives Tobacco Consortium.

- The review panel requested literature from independent sources such as data from ECHA. The process of obtaining the right to data access (right to cite the study) was ongoing at the date of the submission but has been completed shortly after the June 2018 deadline for the priority additive reports. We acknowledge the attempt to obtain data access. In the future, it is advised to assure access before the submission deadline and take ECHA data systematically into account.
- The review panel requested the exact composition of used (reference) cigarettes. We acknowledge the industry for providing the two tables containing tobacco blend ingredients and analysis, respectively.
- The review panel acknowledges the effort to identify pyrolysis products in the chemical analysis. However, a full chemical analysis is desirable for all pyrolysis products, including those for which a validated method was not available. It is unfortunate that validated methods were not developed or implemented for all identified pyrolysis products.
- The explanation of the use of literature data to assess inhalation toxicity, or toxicity of metabolites or pyrolysis products, is acknowledged. However, most of these studies use a

mixture of compounds and therefore the assessment is incomplete.
- The review panel acknowledges the effort to provide pyrolysis products linked to addictiveness.

The JATC WP 9 regrets the clarification to some specific requests.

- The Industry Consortium considered the systematic literature search by KSR was performed following the highest standards including e.g., a study protocol and predefined acceptance/ inclusion criteria. The opinion of the review panel is that many relevant articles from independent sources were not listed. For example, literature studies describing the relationship between the cooling sensation properties and facilitation of deeper inhalation, and the cooling properties of menthol are not included in the industry report (see Report on Menthol).
- We appreciate the clarification for the use of historical variability in reference cigarette data. However, even increases that are smaller than the historic variability can lead to a significant (meaningful) increase in toxicity and are overlooked by the current approach.
- The claim of the Industry Consortium that *Tobacco products are developed for legal age smokers* might be the case, but that doesn't exclude the fact that cigarette products with characterizing flavors are attractive to non-smokers and young people. For example, 19 year old's would have the legal age for (starting) smoking, but are still young and sensitive to developing nicotine addiction.