FOOD INGREDIENTS AND PACKAGING UNIT

SCIENTIFIC PANEL ON FOOD ADDITIVES AND FLAVOURINGS (FAF)

MINUTES OF THE 22ND PLENARY MEETING

Held on 4-6 May 2021
Online meeting

09:30-17:00 on 4th May 2021
09:30-17:00 on 5th May 2021
09:30-13:00 on 6th May 2021

(Agreed by written procedure on 31 May 2021)

Participants

- Panel Members:

  Gabriele Aquilina, Laurence Castle[1], Karl-Heinz Engel[2], Paul Fowler, Maria José Frutos Fernandez, Peter Fürst[3], Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy[4], Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah[5], Dina (Ine) Waalkens-Berendsen, Detlef Wölfle, Matthew Wright and Maged Younes

- Hearing Experts[6]:

  James Kevin Chipman and Riccardo Crebelli participated on 5th May in the agenda item 7.2.

- European Commission and/or Member States representatives:

  DG SANTE (Health and Food Safety), E2 Food processing technologies and novel foods: Guillermo Cardon, Milada Schulzova and Jiri Sochor

- EFSA:

[1] Partial attendance on 5 May AM
[2] Apologies on 4 May AM
[3] Apologies on 6 May
[4] Apologies on 4 May PM and 5 May PM
[5] Participated on 4 May PM and 5 May PM
[6] As defined in Article 15 of the Decision of the Executive Director concerning the selection of members of the Scientific Committee, the Scientific Panels, and the selection of external experts to assist EFSA with its scientific work: http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/expertselection.pdf

FIP Unit: Claudia Roncancio Peña, Stefania Barmaz, Ana Campos Fernandes, Maria Carfi, Consuelo Civitella, Esraa Elewa, Galvin Ndip Eyong, Alessandra Giarola, Federica Lodi, Carla Martino, Ana Maria Rincon, Camilla Smeraldi, Alexandra Tard and Giorgia Vianello

SCER Team New Approaches in Risk Assessment: José Tarazona

SCER Team Scientific Committee: Reinhilde Schoonjans

1. Welcome and apologies for absence

The Chair welcomed the participants in the meeting. No apologies were received for the whole length of the meeting.

2. Adoption of agenda

The agenda was adopted with the inclusion of item n. 8.1.2.2 Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: Part 1, human and animal health.

3. Declarations of Interest of Scientific Panel members

In accordance with EFSA's Policy on Independence[7] and the Decision of the Executive Director on Competing Interest Management[8], EFSA screened the Annual Declarations of Interest filled out by the Working Group members invited to the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process.

4. Agreement of the minutes of the 21st Plenary meeting held on 23-25 March 2021, as online meeting

The minutes of the 21st Plenary meeting held on 23-25 March 2021 were agreed by written procedure on 19 April 2021[9].

5. Report on written procedures since 21st Plenary meeting

No scientific outputs were adopted by written procedure since the last plenary meeting.

6. Scientific topic(s) for discussion

FOOD ADDITIVES

6.1. Scientific opinion on the safety in use of long-chain glycolipids from *Dacryopinax spathularia* as a food additive (EFSA-Q-2020-00433)

[7] http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf

[8] http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf

[9] https://www.efsa.europa.eu/en/events/event/21st-plenary-meeting-faf-panel

The Panel was presented with the draft opinion on the safety in use of long-chain glycolipids from *Dacryopinax spathularia* as a food additive. The FAF Panel discussed the different parts of the document and unanimously adopted the opinion, subject to incorporation of changes as suggested during the meeting.

The final adopted opinion will be available on the Authority's webpage.

6.2. Re-evaluation of thaumatin (E957) (EFSA-Q-2011-00725)

The FAF Panel was presented with the first draft opinion prepared by the WG Sweeteners following the implementation of the two protocols for the re-evaluation of sweeteners, one on the assessment of the hazard identification and characterisation of sweeteners, and the other one focussing on the exposure assessment, previously developed and agreed by the Panel prior to the start of the assessment of the data.
Steer from the Panel was sought with respect to the overall content and format of the opinion (that will serve as a template for the other scientific opinions currently under preparation) and on the approach to be followed with respect to the use of occurrence and consumption data received from the UK after BREXIT. Consistent with the approach that is being followed by other EFSA Panels (e.g. CONTAM Panel), the Panel agreed that occurrence data submitted by the UK before BREXIT will be considered for assessment of exposure since they can still be representative of the food additive levels in the EU, whereas those submitted after BREXIT will be excluded, as done for non-EU countries. Similarly, consumption data from UK will no longer be considered in the exposure assessments.

The FAF Panel discussed and endorsed parts of the draft document related to the assessment of technical data and exposure assessment.

The draft opinion will be further elaborated by the WG Sweeteners, and a revised version will be distributed to the Panel for discussion and possible adoption at a forthcoming plenary meeting.

7. Other scientific topics for information and/or discussion

FOOD FLAVOURINGS

7.1. Questions received from interested parties after the publication of the EFSA guidance on the assessment of smoke flavouring primary products.

Further to the publication on 2nd March 2021 of the EFSA "Scientific Guidance for the preparation of applications on smoke flavourings primary products", the FIP Unit received some questions from interested parties requesting clarifications on certain aspects of the guidance document.

For transparency reasons and for the benefit of all interested stakeholders, the answers provided by EFSA in response to the questions received were discussed during the current plenary meeting and are published in the Annex A below to the minutes.

FOOD ADDITIVES

7.2. Progress update on the re-evaluation of sweeteners. Genotoxicity data available for: Acesulfame K (E 950) (EFSA-Q-2011-00721); Isomalt (E 953) (EFSA-Q-2011-00723); Sucralose (E 955) (EFSA-Q-2011-00724); Neohesperidine DC (E 959) (EFSA-Q-2011-00726); salt of aspartame-acesulfame (E 962) (EFSA-Q-2011-00727); Lactitol (E 966) (EFSA-Q-2011-00728); Xylitol (E 967) (EFSA-Q-2011-00729); Erythritol (E 968) (EFSA-Q-2011-00730); Cyclamates (E 952 i, ii,iii) (EFSA-Q-2011-00733; EFSA-Q-2011-00734; EFSA-Q-2011-00735); Saccharin Na, Ca, K (E 954 i, ii, iii, iv) (EFSA-Q-2011-00736; EFSA-Q-2011-00737; EFSA-Q-2011-00738; EFSA-Q-2011-00739); Neotame (E 961) (EFSA-Q-2011-00740); Maltitol (E 965 i, ii) (EFSA-Q-2011-00755; EFSA-Q-2017-00490)

At the current plenary, the Panel received feedback from the FAF WG Sweeteners, with respect to the ongoing, preliminary assessment of the available genotoxicity data gathered through the public calls for data launched in preparation of the re-evaluation of sweeteners, additional data received from interested business operators and the literature searches performed for the different substances to be re-evaluated. The hearing experts and members of the FAF WG on Sweeteners, James Kevin Chipman and Riccardo Crebelli, joined the current plenary meeting for this agenda point to address questions from the Panel concerning the preliminary assessment of the genotoxicity data available.

It was explained that for two of the sweeteners included in the re-evaluation programme, sorbitols (E 420) and mannitol (E 421), the screening of the available data has not yet been completed owing to the large amount of publications retrieved through the literature searches conducted. The current discussion was focussed on the assessment of the data for the 13 remaining substances. The genotoxicity assessment of thaumatin (E 957) was addressed in a separate agenda item (see 6.2).

For the remaining sweeteners, the Panel was reminded that since their last evaluation either by EFSA or the SCF, new guidance on the genotoxicity assessment of substances has become available (EFSA SC, 2011[10]; EFSA SC, 2017[11]), recommending a set of core tests for the detection of the three important genetic endpoints: gene mutation, structural chromosomal aberrations (i.e. clastogenicity) and numerical chromosome aberrations (i.e. aneugenicity). Moreover, it was brought to the attention of the Panel that a substantial proportion of the available genotoxicity studies were completed prior to the provision of the current OECD test guidelines, thus resulting in limitations for several of the current assessments. Hence, the aim of the preliminary assessment conducted by the WG Sweeteners was to establish whether the available data for each substance would be considered adequate with respect to the current standards or whether the need for additional information considered relevant for the

[10] EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011; 9(9):2379. [69 pp.] https://doi:10.2903/j.efsa.2011.2379

[11] EFSA Scientific Committee, Hardy, A, Benford, D, Halldorsson, T, Jeger, M, Knutsen, HK, More, S, Naegeli, H, Noteborn, H, Ockleford, C, Ricci, A, Rychen, G, Silano, V, Solecki, R, Turck, D, Younes, M, Aquilina, G, Crebelli, R, Gürtler, R, Hirsch-Ernst, KI, Mosesso, P, Nielsen, E, van Benthem, J, Carfì, M, Georgiadis, N, Maurici, D, Parra Morte, J and Schlatter, J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113

genotoxicity assessment had been identified in order to progress with the overall safety assessment.

The preliminary assessment of the available genotoxicity data has highlighted the need for the following additional data to be generated for each of the following food additives:

- **Acesulfame K (E 950)**: in the first instance, data from the basic battery of *in vitro* tests, i.e. bacterial reverse mutation assay (OECD TG 471) and an *in vitro* micronucleus assay (OECD TG 487).

- **Salt of aspartame-acesulfame (E 962)**: data submitted for the food additive acesulfame K (E 950) will be used also for the re-evaluation of the food additive salt of aspartame-acesulfame (E 962).

- **Isomalt (E 953):** in the first instance, data from the basic battery of in vitro tests, i.e. bacterial reverse mutation assay (OECD TG 471) and an in vitro micronucleus assay (OECD TG 487).

- **Sucralose (E 955):** Additional data would be needed to further assess the positive effects observed in vitro (gene mutations for 1,6-DCF and DNA strand breaks for sucralose, both without exogenous metabolic activation), and in accordance with the recommendation of the EFSA SC on the follow-up of in vitro positive results (EFSA, 2011). The data required would be an in vivo Comet assay by the oral route both for the food additive sucralose (E 955) and for its degradation/hydrolysis product 1,6-dichloro-1,6-dideoxyfructose (1,6-DCF). Based on the in vitro evidence for a direct genotoxic mechanism for both substances, and the inconclusive results provided by a previous in vivo comet assay with sucralose, the recommended tissues to be assessed in the new Comet assay are stomach, colon, liver, lung and blood cells.

- **Neohesperidine DC (E 959)**: data from a new *in vitro* micronucleus assay using the cytokinesis block protocol (OECD TG 487).

In the event of positive results in the in vitro micronucleus assay, a staining of the micronuclei with fluorescent in situ hybridisation (FISH) or antikinetochore antibodies (CREST) analysis will be needed to determine the appropriate follow-up for an in vivo study.

- **Neotame (E 961)**: Additional data would be needed to investigate the potential for neotame to induce the formation of micronuclei in mammalian cells in vivo. As no exposure of the target tissue (bone marrow) to the test item was demonstrated under identical conditions to the mouse in vivo micronucleus test.

Alternatively, in the absence of evidence of systemic or bone marrow exposure under identical experimental conditions to the in vivo micronucleus assay in the mouse, a FISH/CREST analysis of the in vitro micronucleus assay will be needed to determine the appropriate follow-up for an in vivo study.

- **Lactitol (E 966):** in the first instance, data from the basic battery of *in vitro* tests, i.e. bacterial reverse mutation assay (OECD TG 471) and an *in vitro* micronucleus assay (OECD TG 487).

- **Xylitol (E 967):** in the first instance, data from the basic battery of *in vitro* tests, i.e. bacterial reverse mutation assay (OECD TG 471) and an *in vitro* micronucleus assay (OECD TG 487).

For all the *in vitro* assays, in the event of positive results obtained in the test, *in vivo* follow-up would be needed in accordance with the 2011 EFSA SC Guidance on genotoxicity and the draft Guidance on aneugenicity, currently scheduled for possible adoption at the coming EFSA Scientific Committee plenary meeting in June 2021.

As a follow-up to this meeting, the Panel agreed to launch an open call for data to invite interested business operators and other interested parties to submit the requested information to fill the data gaps identified during the preliminary assessment of the available data. According to the indicative timelines for submitting additional/supplementary information to EFSA during the risk assessment, included in the Annex to the EFSA "Administrative guidance for the processing of applications for regulated products"[12], a period of 6 months should be sufficient to generate and provide the additional data needed.

The Panel noted that assessment of these food additives will continue with respect to the re-evaluation of all the other data available (i.e. technical, occurrence, biological and toxicological data). However, the scientific opinions cannot be finalised in the absence of conclusions on the genotoxic potential of the substances. The Panel is aware that according to Regulation (EC) No 257/2010, the re-evaluation of sweeteners should have been completed by the 31st December 2020; however, owing to the past and current workload of the Panel and the newly identified need for additional request for data, the Panel anticipated a further delay in the completion of the safety assessment of sweeteners.

Moreover, the Panel discussed and agreed on the following approach for the remaining sweeteners to be re-evaluated:

- **Salt of aspartame-acesulfame (E 962)**: the assessment will be based on the read-across from the data on the two components aspartame (E 961) and acesulfame K (E 950). While for the latter the genotoxicity assessment is still ongoing, the former has been re-evaluated by the ANS Panel in 2013. New data from the literature covering the timespan since the re-evaluation will be gathered and, if relevant, included in the present re-evaluation of E 962.

For the following substances, the available studies and potential data gaps are still being assessed according to the protocol, before preliminary conclusions with respect to the need for additional data are drawn:

- **Cyclamates (E 952 i, ii,iii)**
- **Saccharins (E 954 i, ii, iii, iv)**
- **Erythritol (E 968)**
- **Maltitols (E 965 i, ii)**

As the assessment of the data by the Working Group progresses, the Panel will be presented with an update at forthcoming plenary meetings and will decide on the need for additional data to be requested also for the other sweeteners under re-evaluation.

8. Feedback from the Scientific Committee/Scientific Panels, EFSA, the European Commission

8.1. Scientific Committee and Scientific Panel(s) including their Working Groups

[12] https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2018.EN-1362

The Chair provided general feedback from the last meeting of the Scientific Committee which took place on 14-15 April 2021.

8.1.1. FAF Panel Working Groups

8.1.1.1 FAF WG Food Additives Applications

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the **minutes of the WG**.

8.1.1.2 FAF WG on the re-evaluation of miscellaneous food additives

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the **minutes of the WG**.

8.1.1.3 FAF WG on the re-evaluation of food additives permitted in foods for infants below 16 weeks of age

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the **minutes of the WG**.

8.1.1.4 FAF WG on the re-evaluation of remaining food additives other than colours and sweeteners

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the **minutes of the WG**.

8.1.1.5 FAF WG on Sweeteners

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the **minutes of the WG**.

8.1.1.6 FAF WG on Flavourings

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the **minutes of the WG**.

8.1.1.7 FAF WG on Specifications of Food Additives

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the **minutes of the WG**.

8.1.1.8 FAF WG on Guidance Update on Flavourings

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the **minutes of the WG**.

8.1.2. EFSA Scientific Commitee

8.1.2.1 EFSA scientific opinion on the guidance on technical requirements of regulated food and feed product applications to establish the presence of particles in the nanoscale (EFSA-Q-2019-00692)

8.1.2.2 Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: Part 1, human and animal health (EFSA-Q-2020-00269)

The Panel received a presentation on practical cases and experience gathered from different assessments across EFSA that were taken into account for the elaboration of the two draft EFSA Scientific Committee guidance documents, applicable to the assessment of particles in the nanoscale. Feedback from the Panel was sought on the

two documents before their finalisation and adoption by the EFSA Scientific Committee, currently scheduled by the end of June 2021.

8.2. EFSA including its Working Groups/Task Forces

The Panel was presented with a tentative workplan for the rest of the year, elaborated on the basis of the information from the different FAF Panel Working Groups.

8.3. European Commission

This agenda item was not discussed due to lack of time.

9. New mandates

The Panel was informed of the following mandates received from the European Commission since the 21st Plenary meeting held in March:

- **Request for EFSA to perform a risk assessment and to provide a scientific opinion on the safety in use of jagua (genipin-glycine) blue as a food additive** - **EFSA-Q-2021-00231**

  This new mandate (M-2021-00559) from the European Commission covers the request for evaluation of an application for the proposed new food additive jagua (genipin-glycine) blue as a food additive (EFSA-Q-2021-00231) and is under consideration by EFSA.

  Pending confirmation of the validity of the application, the scientific assessments will be carried out by the Panel WG Food Additives Applications.

In addition, the Panel discussed the following mandates which were briefly presented during previous plenary meetings but not discussed due to lack of time:

- **Request for an updated scientific opinion from the European Food Safety Authority as regards the safety of sulphur dioxide (E 220), sodium sulphite (E 221), sodium bisulphite (E 222), sodium metabisulphite (E 223), potassium metabisulphite (E 224), calcium sulphite (E 226), calcium bisulphite (E 227) and potassium bisulphite (E 228) as food additives** – **EFSA-Q-2021-00180**

  As anticipated at the previous plenary meeting, EFSA has received a mandate from the EC requesting to provide an updated scientific opinion as regards the safety of the food additives sulphur dioxide (E 220), sodium sulphite (E 221), sodium bisulphite (E 222), sodium metabisulphite (E 223), potassium metabisulphite (E 224), calcium sulphite (E 226), calcium bisulphite (E 227) and potassium bisulphite (E 228).
  In particular, EFSA is requested to re-evaluate the database and the temporary group ADI for the food additives sulphur dioxide–sulphites (E 220–228), as well as to refine the exposure assessment for these food additives, taking into account the data submitted by business operators in reply to the call for data issued by the Commission, as well as any new relevant data retrieved from the published literature and the conclusions from the currently ongoing scientific assessment of ECHA on sulphur dioxide. In accordance with the provisions of Article 30 of Regulation (EC) No 178/2002, EFSA is requested to identify potentially contentious scientific issues with the work of ECHA and to cooperate with ECHA with a view to either resolving the divergence or presenting a joint document to the Commission clarifying the contentious scientific issues and identifying the relevant uncertainties in the data.

The requested scientific opinion should be delivered by February 2022. This period should be, in any case, aligned with the ongoing ECHA evaluation procedures and could be extended if required.

The Panel considered that none of the existing Working Groups has the required expertise and remit to carry out the assessment of the data described under the terms of reference and therefore a new Working Group should be established.

- **Request for an updated scientific opinion from the European Food Safety Authority as regards the safety of iron oxides and hydroxides (E 172) as food additives – EFSA-Q-2021-00178**

  As anticipated at the previous plenary meeting, EFSA has received a mandate from the EC requesting to provide an updated scientific opinion on the safety of iron oxides and hydroxides (E 172) as food additives. The terms of reference of this new mandate cover, both i) the assessment of the analytical data provided by interested business operators in support of proposed amendment of the specifications of the food additives iron oxides and hydroxides (E 172), with respect to the introduction of separate entries according to their colour (yellow iron oxide, red iron oxide and black iron oxide), the inclusion of additional parameters related to the particle size and particle size distribution, and a revision of the limits for the impurities of toxic elements and ii) an assessment of the toxicity database in support of the safety of the proposed amendments to the specifications of the food additives iron oxides and hydroxides (E 172) with the aim of establishing health-based guidance values for the individual substances (yellow iron oxide, red iron oxide and black iron oxide) or the group of substances, as deemed appropriate by the scientific evidence. In particular, EFSA is requested to re-evaluate the database for the food additives iron oxides and hydroxides (E 172) taking into account the data submitted by business operators in reply to the call for data issued by the European Commission, as well as any new relevant data retrieved from the published literature. EFSA should also consider whether, in the light of the physico-chemical characterisation of the materials used as the food additive E 172, the data requirements specified in the "EFSA Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain" or any updated EFSA Scientific Committee guidance, would be applicable. The requested scientific opinion should be delivered within 12 months from the receipt of the mandate (i.e. by February 2022). This period may be extended in the event that additional information would need to be sought from the relevant interested parties during the assessment.

  The Panel agreed on a stepwise approach to address this new mandate and considered that the existing WG Specifications has the required expertise and remit to carry out the assessment of the data described under the first point of the terms of reference, that will be addressed as the starting point of the assessment.

- **Request for a scientific opinion from the European Food Safety Authority as regards the safety of indigotine, indigo carmine (E 132) containing not less than 85% total colouring matters, calculated as the sodium salt, as a food additive, as well as on the specifications for E 132** – **EFSA-Q-2021-00180**

  As anticipated at the previous plenary meeting, EFSA has received a mandate from the EC requesting to provide an updated scientific opinion on the safety of indigotine, indigo carmine (E 132) containing not less than 85% total colouring matters, calculated as the sodium salt, as a food additive, as well as on the specifications for E 132. The terms of reference of this new

mandate cover, both i) the assessment of the new technical data provided by interested business operators to support the proposed amendment of the specifications of the food additive indigotine, indigo carmine (E 132) and ii) an assessment of the new toxicity data on the safety of indigotine, indigo carmine (E 132) containing not less than 85% total colouring matters, calculated as the sodium salt, as a food additive.
The requested scientific opinion should be delivered within 12 months from the receipt of the mandate (i.e. by March 2022). This period may be extended in the event that additional information would need to be sought from the relevant interested parties during the assessment.

The Panel agreed on a stepwise approach to address this new mandate and considered that the existing WG Specifications has the required expertise and remit to carry out the assessment of the data described under the first point of the terms of reference, that will be addressed as the starting point of this assessment.

10. Any Other Business

The Panel was informed that on the next plenary meeting (currently planned on 22-24 June) there will be the election of the Chair and Vice-Chairs. Experts were invited to express their interest to run for the election to the Unit in advance of the plenary meeting.

Annex A

Questions and answers on the EFSA Scientific Guidance for the preparation of applications on smoke flavouring primary products

Version 1 – discussed by Food Additives and Flavourings (FAF) Panel at its 22nd Plenary meeting held on 04-06 May 2021

The answers provided to the questions listed below are without prejudice to the final decisions that EFSA may reach in future evaluations of smoke flavouring primary products.

Characterisation of smoke flavouring primary products

1. **From an analytical point of view, it is still not very clear how the "tentatively identified fraction" and "tentatively identified molecules" will be treated and assessed. Most components of smoke flavours are not commercially available and therefore cannot be used for the comparison of chromatographic and mass spectral data. This will lead to numerous identified "tentatively" components.**

EFSA's answer to Q.1:

As depicted in Figure C.1 of the FAF Panel Scientific Guidance for the preparation of applications on smoke flavouring primary products, the genotoxicity assessment of smoke flavouring primary products is a two-step approach consisting of (i) conclusions on all identified components regarding their genotoxic potential and (ii) genotoxicity testing of the unidentified part of the Primary Product. The first step requires unequivocal chemical identifications of the individual components.

"Tentatively" identified components should be considered as part of the unidentified fraction of the primary product. As stated in the guidance document (section 1.2.4.4), any analytical information available to characterise the type and to estimate the proportions of chemical classes constituting the unidentified fraction should be presented. To this end, it is requested in the guidance document that the criteria underlying the tentative identifications should be clearly described (section 1.2.4.3.1). The more substantiated "tentative" identifications of components are, the more this information will assist in the assessment of the unidentified fraction.

2. **The guidance states: "*if the detailed chemical analysis reveals changes in the chemical composition as a result of the modifications of the production process, this triggers the need for the submission of a new application.*" Hence, there is a significant risk, if EFSA decides during evaluation that a new application is demanded.**

EFSA's answer to Q.2:

If the production process of an authorised primary product is modified, it is the responsibility of the applicant to assess the potential impact of these modifications on the chemical composition of the primary product and its compliance with existing specifications. As mentioned in the EFSA guidance on smoke flavourings, *"if the existing specifications of a primary product are not met or if the detailed chemical analysis reveals changes in the chemical composition as a result of the modifications of the production process, this triggers the need for the submission of a new application."*

Proposed uses and exposure assessment

3. **The new 'EFSA exposure tool' mentioned in the EFSA guidance in section 2.2 is not available. Hence it is unclear how exposure assessment will be conducted. In addition, the FAIM model will lead to a lot of uncertainty and anticipated overestimation of exposure.**

EFSA's answer to Q.3:

The FAIM tool is based on the food categories specified in Annex II, Part D of Regulation (EC) No 1333/2008[13]. This food categories nomenclature should also be referred to for the smoke flavourings. However, FAIM tool contains more detailed food categories that could be used with respect to those mentioned in the current legislation indicating the authorised uses of smoke flavourings (see Annex to Commission Implementing Regulation (EU) No 1321/2013). Despite this, FAIM might still provide an overestimation of the dietary exposure as all foods within a food category are considered to contain the smoke flavouring primary product at the provided use level(s). This is mentioned in the guidance.

The Dietary Exposure (DietEx) tool (i.e. the new EFSA exposure tool) is expected to be released by June 2021 at the following link: https://www.efsa.europa.eu/en/science/tools-and-resources. This tool is expected to complement results obtained with the FAIM tool, since it will allow the use of more specific food categories through the FoodEx2 classification system. Estimates of exposure will therefore be more accurate if the submitted use and use levels will be provided for detailed food categories. However, in this case, food categories will not be aligned with those specified in the food categories nomenclature used for smoke flavourings.

The exposure assessment will be conducted by EFSA as described in the guidance document, see section 2.2. Thus, applicants should provide estimates with both tools: FAIM (mandatory) and DietEx (optional). In case needed, EFSA might still refine the assessment by selecting the very specific foods from the Comprehensive Database (e.g. through the use of facets) that will not be available in the DietEx tool.

Safety data

4. **What is the recommended maximum dose to be used in *in vivo* genotoxicity studies of complex mixtures such as smoke flavouring primary products?**

EFSA's answer to Q.4:

As mentioned by EFSA in the recently published technical report "Outcome of the public consultation on the draft scientific guidance for the preparation of applications on smoke flavouring primary products" (see Table 2, reply to question #21, pages 38-39), the range of concentrations or doses used in *in vivo* genotoxicity tests, from a maximum tolerated dose (MTD) to a dose producing little or no toxicity, should be established on the basis of the results of a preliminary range-finding study. This is in line with the recommendations given in OECD test guidelines.

[13] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354, 31.12.2008

Furthermore, in both OECD TG 474[14] and OECD TG 489[15] it is reported: "*If the test chemical does not produce toxicity in a range-finding study or based on existing data, the highest dose for an administration period of 14 days or more should be 1000 mg/kg body weight/day, or for administration periods of less than 14 days, 2000 mg/kg/body weight/day. However, if the test chemical does cause toxicity, the MTD should be the highest dose administered and the dose levels used should preferably cover a range from the maximum to a dose producing little or no toxicity. For certain types of test chemicals (e.g. human pharmaceuticals) covered by specific requirements, these limits may vary.*"

In the case of smoke flavourings primary products, EFSA is of the view that if no toxicity is observed in an appropriately designed range-finding study, it would be appropriate to test higher doses than the above-mentioned maximum limits, in order to increase the dose of each of the individual components. If this resulted in toxicity, the corresponding dose would be considered sufficiently high.

However, in the absence of any toxicity, the highest dose to be applied is limited by the maximum volume that should be given to rodents. According to OECD TG 474 and 489, the maximum volume of liquid that can be administered by gavage at one time should not normally exceed 1 mL/100 g body weight except in the case of aqueous solutions where a maximum of 2 mL/100 g may be used.

5. **The guidance provides a list of additional endpoints indicative for effects on the immune system to be assessed in the context of the 90-day oral toxicity study, to be conducted in line with OECD TG 408[16]. Most of these parameters have never been used in regulatory rat studies under Good Laboratories Practices (GLP). Would it be possible to receive some explanation why all these parameters were added and receive some guidance and practical input on their assessment?**

EFSA's answer to Q.5:

The additional immunological parameters were added to the standard OECD TG 408 repeated dose 90-day oral toxicity study for renewal applications, to allow a full investigation of the potential effects on the immune system for the tested primary product. This option has been considered, following the comments from interested parties received during the public consultation of the draft guidance (see EFSA's responses to comments #5 and #27 in Table 2 of the "Outcome of the public consultation on the draft scientific guidance for the preparation of applications on smoke flavouring primary products"). Following these comments, EFSA's experts reconsidered the toxicological dataset originally requested for renewal applications, which included a full Extended One-Generation Reproductive Toxicity Study (EOGRTS), as currently required for applications for new smoke flavourings. Although, the EOGRTS remains the preferred option for renewals, an alternative option was considered appropriate by the EFSA's experts who developed the guidance, consisting of a 90-day toxicity study (OECD TG 408), with the additional parameters for the

[14] OECD (Organisation for Economic Co-operation and Development), 2016. Test No. 474: Mammalian erythrocyte micronucleus test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264762-en

[15] OECD (Organisation for Economic Co-operation and Development), 2016. Test No. 489: In vivo mammalian alkaline comet assay, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264885-en

[16] OECD (Organisation for Economic Co-operation and Development), 2018. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents (OECD TG 408), in Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Publishing, Paris, https://doi.org/10.1787/9789264304741-23-en

assessment of immunotoxicity, plus an OECD TG 414[17] prenatal developmental toxicity study in rats. This alternative has the advantage of (1) accommodating the time scale issue linked to Art 12 of Regulation (EC) No 2065/2003[18] and (2) allowing, at the same time, the identification of potential neurotoxic, endocrine, immunological (given the additional immunotoxicity parameters) and developmental effects.

The issue related to the need for developing and validating these endpoints according to GLP rules, e.g. the lack of historical control data, is acknowledged. However, while historical control data may be helpful for the interpretation of study results, the basis for the identification of treatment-related effects will in principle reside with the concurrent controls.
The methods for investigating these parameters have been in use for decades and their implementation in CRO laboratories should be feasible. As mentioned in the guidance document (section 3.3.3): *"some guidance for investigating these additional parameters may be found for example in 'Methods in Immunotoxicology' (Burleson et al., 1995), or in 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) or in the WHO/IPCS Guidance for immunotoxicity risk assessment for chemicals (WHO/IPCS, 2012)."*
As an additional guidance on where methodological input on the investigation of these endpoints can be found, please consider the following:

"At term (at sacrifice):
- *Histopathology (lymphatic organs(*)), including bone marrow cellularity;*
- *Weighing lymphoid organs(*)*

(*) Standard parameters in OECD TG 408."

Regarding the investigation of bone marrow cellularity, the preferred method is to measure the number of cells in the suspensions prepared from bone marrow. Cell count data can be evaluated in conjunction with the histological examination of the bone marrow to judge effects of test articles on the hematopoietic system. However, examination of smears by an experienced pathologist may also provide sufficient information.

"In blood:
- *Immunoglobulin isotypes*
- *Complement assays: total serum haemolytic activity or individual components*
- *C-reactive protein (CRP)*
- *Total and differential white blood cell count (*)"*

Kits are commercially available from different suppliers to investigate the above parameters.

Regarding the investigation of immunoglobulin isotypes, it is suggested to start with total IgG, IgM, IgA and IgE. If changes in total IgG are observed, it is recommended to evaluate IgG isotypes. For the complement assays, typically, C3 and C4 proteins should be measured. These complement markers should provide sufficient evidence of effects on the complement system.

"In spleen:"
In rodents, the analysis of leukocyte subpopulations and the functional tests as described below and in the EFSA guidance, are done typically using the spleen as a source of cells and not on peripheral blood. The issue with peripheral blood is that the amount of blood and cells that can be obtained from an animal will likely be insufficient. However, it may be considered acceptable to conduct some of these analyses, in blood rather than in spleen if the number of cells is enough.

[17] OECD (Organisation for Economic Co-operation and Development), 2018. Test No. 414: Prenatal Developmental Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070820-en

[18] Regulation (EC) No 2065/2003 of the European Parliament and of the Council of 10 November 2003 on smoke flavourings used or intended for use in or on foods. OJ L 309, 26.11.2003, p. 1–8.

• Phenotypic analysis of spleen cells (CD4 and CD8 T cells, regulatory T cells, B cells, natural killer (NK) cells, macrophages)"
Flow cytometry (FACS analysis) is a routine test, specific antibodies are available from different suppliers. In the case of rodents, typically this analysis is performed using splenocytes. See Chapter 12 of 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) for details.

"• *Natural killer cell functional analysis"*
See Chapter 15 of 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) for details. Please consider that both the number and the functionality of the NK cells need to be investigated to get an indication of any potential alterations.

"• *Phagocytic activity*"
This parameter may also be evaluated using splenocytes, see Chapter 17 of 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) for details. Kits are commercially available.

"• *Mitogen stimulation assays for B and T cells*"
See Chapter 14 of 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) for details. It is considered enough to measure cell proliferation after mitogen stimulation as this would provide an indication of the ability of B and T cells to undergo clonal expansion, which is central in the initial phase of the activation of acquired immunity. In case the applicant is interested in investigating the mechanisms of immunotoxicity, it may be advisable to analyse additional parameters, such as cytokine release or surface marker expression.

6. **T-cell dependent antibody response (TDAR) assay is considered as a gold standard in the field of immunotoxicology to evaluate the humoral immune response to a T-cell dependent antigen. Kinetics of IgG and IgM against the T-cell dependent antigen can be used to evaluate any possible effects on the immunosystem. Is this test recommended by EFSA?**

EFSA's answer to Q.6:

TDAR analysis is indeed a gold standard in immunotoxicology. In fact in the EFSA guidance on smoke flavouings it is stated that the preferred option to evaluate the safety of smoke flavouring primary products is to conduct an Extended One Generation Reproductive Toxicity study (EOGRTS), according to OECD TG 443[19], including a cohort specifically targeted to investigate the potential immunotoxicity of a test item, where TDAR assay is the prime element. However, in case an EOGRT study cannot be conducted due to timeline issues applicable to renewal applications, an alternative option has been recommended by EFSA which consists in a 90-study with the additional investigation of a wide range of immunological parameters, not foreseen in the EOGRT study protocol. This was dictated by an attempt to obtain as much information as possible on potential immunotoxicity from a subchronic oral toxicity study, without involving the use of additional animals, in line with the 3R principle.

7. **In the EFSA guidance on smoke flavourings it is mentioned "*A new 90-day oral toxicity study may be submitted according to the latest version of OECD TG 408[5] (OECD, 2018a), including the assessment of neurotoxicity, since in the 90-day studies already available from previous submissions neurotoxicity was either not***

[19] OECD (Organisation for Economic Co-operation and Development), 2018. Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264185371-en

***included or inadequately addressed*". It is assumed that this refers to the standard neurotoxicity parameters already included in the OECD TG 408 and no additions are needed. Is that correct?**

EFSA's answer to Q.7:

Yes, this is correct. The standard neurotoxicity parameters included in OECD TG 408 repeated dose 90-day oral toxicity study are considered sufficient in this case, i.e. sensory reactivity to stimuli of different types such as auditory, visual and proprioceptive stimuli (functional observational battery (FOB)).

8. **The guidance also mentions "*This new oral 90-day toxicity study should also include a full assessment of parameters indicative of possible effects on the endocrine system as specified in Annex B of OECD TG 408*". Does this refer to the 'required measures' only or also the 'optional measures'?**

EFSA's answer to Q.8:

The parameters to be assessed for the detection of possible effects on the endocrine system are the ones specified in Annex B of OECD TG 408, including both the 'required' and the 'optional measures'.

Uncertainty

9. **It is still unclear how EFSA will apply uncertainty and what will be the impact on the overall safety assessment of the smoke flavouring primary products.**

EFSA's answer to Q.9:

This question concerns uncertainty in future assessments of individual smoke flavouring primary products. Standard uncertainties (as listed in Appendix G of the EFSA guidance) require no further assessment as they have been considered by the Panel when developing the guidance document. Non-standard uncertainties will be identified by EFSA when conducting the assessment, following the criteria described in Appendix G of the guidance. When non-standard uncertainties are present, the combined impact of the non-standard uncertainties will be assessed by EFSA based on the available evidence plus expert judgement, following the approach outlined in section 4.2 of the 2018 EFSA Guidance on Uncertainty Analysis in Scientific Assessments , and will be considered in EFSA's overall assessment of whether the smoke flavouring primary product achieves the level of safety required by the EFSA guidance[1]. As explained in section 4.3 of the guidance document, applicants can contribute in reducing the uncertainties in the assessment by providing comprehensive information on all aspects of the risk assessment and doing every effort to fulfil the requirements as laid down in the guidance, using state-of-the-art approaches.

Timelines for submission of data

10. **Considering the additional studies that have been requested in the updated EFSA's guidance document, it may be challenging for applicants to meet the submission deadline applicable to renewal applications of smoke flavourings primary products, as foreseen by Regulation (EC) No 2065/2003. Would it be possible to delay the deadline of 30 June 2022?**

EFSA's answer to Q.10:

The deadline to submit renewals applications is set by the legislation. EFSA is not in the position to grant any extension being not responsible for the application of European Law. On this aspect, please contact the European Commission[20].

Note

It is reminded that EFSA has implemented several initiatives to support applicants in understanding the evaluation process of applications for regulated products and to engage with them during all phases of the life-cycle of applications, including pre-submission activities such as general pre-submission advice and renewal pre-submission advice.
For the different possibilities of interaction with EFSA in the different phases of the application life-cycle, please consult EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products.

[20] https://ec.europa.eu/info/departments/health-and-food-safety_en#contact