SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 28 September 2023

doi: 10.2903/j.efsa.2023.8364

# Scientific opinion on the renewal of the authorisation of Zesti Smoke Code 10 (SF-002) as a smoke flavouring Primary Product

EFSA Panel name on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Gisela Degen, Karl-Heinz Engel, Paul J Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Matthew Wright, Romualdo Benigni, Polly Boon, Claudia Bolognesi, Eugenia Cordelli, Kevin Chipman, Ullrika Sahlin, Maria Carfì, Carla Martino, Salvatore Multari, Vasantha Palaniappan, Alexandra Tard and Wim Mennes

## Abstract

The EFSA Panel on Food Additives and Flavourings (FAF) was requested to evaluate the safety of the smoke flavouring Primary Product Zesti Smoke Code 10 (SF-002), for which a renewal application was submitted in accordance with Article 12(1) of Regulation (EC) No 2065/2003. This opinion refers to the assessment of data submitted on chemical characterisation, dietary exposure and genotoxicity of the Primary Product. Zesti Smoke Code 10 is obtained by pyrolysis of hickory and oak woods. Given the limitations of the quantification approach employed by the applicant, the Panel could not judge whether the applied methods meet the legal quality criterion that at least 80% of the volatile fraction shall be identified and quantified. At the maximum proposed use levels, dietary exposure estimates calculated with DietEx ranged from 0.02 to 4.6 mg/kg body weight (bw) per day at the mean and from no dietary exposure to 13.0 mg/kg bw per day at the 95th percentile. The Panel concluded that four components in the Primary Product raise a potential concern for genotoxicity. In addition, a potential concern for genotoxicity was identified for the unidentified part of the mixture. The Primary Product contains furan-2(5*H*)-one and benzene-1,2-diol, for which a concern for genotoxicity was identified *in vivo* upon oral administration. Considering that the exposure estimates for these two components are above the threshold of toxicological concern (TTC) of 0.0025 μg/kg bw per day for DNA-reactive mutagens and/or carcinogens, the Panel concluded that the Primary Product raises concern with respect to genotoxicity.



**Keywords:** Zesti Smoke Code 10, SF-002, smoke flavouring Primary Product, genotoxicity, furan-2(5*H*)-one, benzene-1,2-diol, catechol

**Requestor:** European Commission

**Question number:** EFSA-Q-2022-00415

**Correspondence:** fip@efsa.europa.eu

**Panel members:** Maged Younes, Gabriele Aquilina, Laurence Castle, Gisela Degen, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen and Matthew Wright.

**Declarations of interest:** If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

**Acknowledgements:** The Panel wishes to thank the following for the support provided to this scientific output: Edoardo Carnesecchi, Gabriele Gagliardi, Agnieszka Mech, Ana Rincon, Alkiviadis Stagkos-Georgiadis, the members of the SCER Cross-cutting WG nanotechnologies: Maria Bastos, Jacqueline Castenmiller, Francesco Cubadda, Mohammad Chaudhry, Roland Franz, David Gott, Maria Henriqueta Louro, Jan Mast, Alicja Mortensen, Agnes Oomen, Eveline Verleysen, Stefan Weigel. Furthermore, the Panel wishes to thank the following for the preparatory work performed for the genotoxicity assessment of the Primary Product: Arianna Bassan (Section 3.4.1), Manuela Pavan (Section 3.4.1) and Joanne Gartlon (Section 3.4.2).

**Suggested citation:** EFSA FAF Panel (EFSA Panel name on Food Additives and Flavourings), Younes, M., Aquilina, G., Castle, L., Degen, G., Engel, K.-H., Fowler, P. J., Frutos Fernandez, M. J., Fürst, P., Gundert-Remy, U., Gürtler, R., Husøy, T., Manco, M., Moldeus, P., Passamonti, S., Shah, R., Waalkens-Berendsen, I., Wright, M., Benigni, R., … Mennes, W. (2023). Scientific opinion on the renewal of the authorisation of Zesti Smoke Code 10 (SF-002) as a smoke flavouring Primary Product. *EFSA Journal*, *21*(11), 1–62. https://doi.org/10.2903/j.efsa.2023.8364

**ISSN:** 1831-4732

## Table of contents

# 1. Introduction

## 1.1. Background and Terms of Reference as provided by the requestor

### 1.1.1. Background

Regulation (EC) No 2065/2003[1] establishes a procedure for the safety assessment and the authorisation of smoke flavouring primary products with a view to ensuring a high level of protection of human health and the effective functioning of the internal market. No smoking flavouring or any food where such a smoking flavouring is present (in or on) can be placed in the market if the smoke flavouring is not an authorised primary product or is not derived therefrom and if the conditions of use laid down in the authorisation in accordance with this Regulation are not adhered to (Article 4 (2) of Regulation (EC) No 2065/2003).

Commission Implementing Regulation (EU) No 1321/2013[2] authorised 10 smoke flavouring primary products for a 10-year period, due to expire on 31 December 2023.

The European Commission has received an application for the renewal of the authorisation of the smoke flavouring primary product Zesti Smoke Code 10 (SF-002) for a 10-year period, in accordance with Article 12 of Regulation (EC) No 2065/2003.

### 1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to evaluate the safety of the smoke flavouring primary product Zesti Smoke Code 10 (SF-002), for which a renewal application has been submitted, in accordance with Article 12 of Regulation (EC) No 2065/2003.

The safety assessment shall be carried-out in two steps. Firstly, EFSA shall give a scientific opinion on the data included in the renewal application dossier related to the chemical characterisation, the genotoxicity and the dietary exposure of Zesti Smoke Code 10 (SF-002).

Secondly, provided that the genotoxic concern can be ruled out in the first part of the evaluation, EFSA shall complete the rest of the safety assessment without delay upon submission of the relevant pending data from the applicant.

## 1.2. Interpretation of the Terms of Reference

In line with the terms of reference (see Section 1.1.2), the safety of the Primary Product will be assessed in two steps.

The current (first) opinion will address the chemical characterisation, genotoxicity and dietary exposure to the smoke flavouring Primary Product.

If in the first opinion, no concern for genotoxicity is raised, EFSA will issue a second opinion assessing the toxicity other than genotoxicity data, as required by the EFSA guidance for the preparation of applications on smoke flavouring Primary Products (EFSA FAF Panel, 2021).

## 1.3. Additional information

EFSA issued a previous opinion on the safety of this smoke flavouring Primary Product Zesti Smoke Code 10 in 2009 (EFSA CEF Panel, 2009), updated in 2011 (EFSA CEF Panel, 2011).

Following the safety assessment from EFSA, Zesti Smoke Code 10 was authorised in the European Union and assigned the unique code 'SF-002', according to Commission Implementing Regulation (EU) No 1321/2013, establishing the Union list of authorised smoke flavouring Primary Products, for a 10-year period with effect from 1 January 2014.

The present opinion refers to an assessment of the data submitted by the authorisation holder for the renewal of the authorisation of Zesti Smoke Code 10 (SF-002) as a smoke flavouring Primary Product, in line with Article 12(1) of Regulation (EC) No 2065/2003.

[1] 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.

[2] Commission Implementing Regulation (EU) No 1321/2013 of 10 December 2013 establishing the Union list of authorised smoke flavouring primary products for use as such in or on foods and/or for the production of derived smoke flavourings. OJ L 333, 12.12.2013, p. 54–67.

# 2. Data and Methodologies

## 2.1. Data

The present evaluation is based on the data provided by the applicant in the form of a technical dossier, submitted according to Article 12(1) of Regulation (EC) No 2065/2003 for the renewal of the authorisation of the smoke flavouring Primary Product Zesti Smoke Code 10 (SF-002).

In accordance with Article 38 of the Regulation (EC) No 178/2002[3] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation and of the Decision of the EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[4] the non-confidential version of the dossier is published on Open.EFSA.[5]

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the application from 3 March to 24 March 2023, for which no comments were received.

Additional information was sought from the applicant during the assessment process by requests from EFSA sent on 24 November 2022 and was subsequently provided (see Documentation provided to EFSA No. 2).

The Panel acknowledged the submission of data on toxicity other than genotoxicity by the applicant in the technical dossier (see Documentation provided to EFSA No. 1 and 3). As indicated in Section 1.2, the assessment of these data is outside the scope of the present opinion.

## 2.2. Methodologies

The safety assessment of the Primary Product Zesti Smoke Code 10 (SF-002) was conducted in line with the requirements laid down in Regulation (EC) No 2065/2003 and following the principles of the EFSA guidance for the preparation of applications on smoke flavouring Primary Products (EFSA FAF Panel, 2021).

The principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) as well as the relevant cross-cutting guidance documents from the EFSA Scientific Committee published after the adoption of the guidance on smoke flavourings (EFSA FAF Panel, 2021), in particular the 'Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles' (EFSA Scientific Committee, 2021a), were also considered during the risk assessment.

The uncertainty analysis was performed by checking whether standard or non-standard sources of uncertainties are present, as outlined in the standard procedure described in Section 4.2 of the EFSA guidance on smoke flavouring and listed in Table G.1 therein (EFSA FAF Panel, 2021). Standard uncertainties are not discussed in detail in the present assessment. In case of the presence of non-standard uncertainties, these are reported in the relevant sections of the opinion and their combined impact on the assessment was evaluated by the Panel (see Section 4).

# 3. Assessment

## 3.1. Technical data

### 3.1.1. Manufacturing process

#### 3.1.1.1. Source materials for the Primary Product

The source material of Zesti Smoke Code 10 is hardwood sawdust from 50 to 60% hickory (*Carya ovata*) and from 40 to 50% white oak (*Quercus alba*) (Documentation provided to EFSA No. 1).

[3] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[4] Decision available online:https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[5] The non-confidential version of the dossier, following EFSA's assessment of the applicant's confidentiality requests, is published on Open.EFSA and is available at the following link: https://open.efsa.europa.eu/dossier/SFL-2022-7010

According to the applicant, the wood is not subjected to any chemical treatment, including treatment with pesticides.

#### 3.1.1.2. Method of manufacture of the Primary Product

Dried wood sawdust is subjected to pyrolysis in a rotary kiln and the formed smoke vapour is condensed. The condensate is held in a storage tank for 10 days to induce phase separation and the insoluble high density tar fraction is continuously removed. The remaining aqueous smoke condensate is filtered (1 μm) and subjected to an internal quality control (acidity; refraction index as °BRIX). According to this procedure, aqueous smoke condensates are manufactured at two production sites. In the final step, these aqueous condensates are combined and subjected to an additional settling in a tank for 1–2 days. The Primary Product Zesti Smoke Code 10 is obtained after decantation and subjecting the aqueous condensate to a filtration step (1 μm).

The applicant submitted a description of the manufacturing process, with information on the drying step of the sawdust and the pyrolysis conditions.

### 3.1.2. Identity of the Primary Product

#### 3.1.2.1. Trade name of the Primary Product

The trade name of the Primary Product is Zesti Smoke Code 10.

#### 3.1.2.2. Information on existing evaluations from other regulatory bodies and authorisations in non-EU countries

The applicant indicated that the smoke flavouring Zesti Smoke Code 10 has not been evaluated by regulatory bodies other than EFSA.

Regarding the existing authorisations in non-EU countries, the applicant stated that Zesti Smoke Code 10 is currently authorised in the United Kingdom, Canada, Australia and New Zealand, the United States, Japan, Korea, China and Indonesia (Documentation provided to EFSA No. 1).

#### 3.1.2.3. Description of the physical state and sensory characteristics

The applicant described the smoke flavouring Primary Product as '*an aqueous amber brown liquid with characteristics of smoke aroma and flavour*.' The Primary Product has a pH ranging from 2.0 to 2.5, a viscosity (at 25°C) ranging from 2 to 3 Cp, a staining index (at 440 nm) ranging from 70 to 100, a refraction index ranging from 28 to 33 °BRIX and a density (at 20°C) of approximately 1,070 g/L (Documentation provided to EFSA No. 1 and 2). The applicant described the Primary Product as '*miscible in alcohol-based solvents and immiscible in oil-based solvents*' (Documentation provided to EFSA No. 1).

#### 3.1.2.4. Chemical composition of the Primary Product

The compositional data for the Primary Product, provided by the applicant in the original dossier and in response to an EFSA request for additional information (Documentation provided to EFSA No. 1 and 2), are summarised in Table 1. Although the applicant was requested to provide compositional data for more than one batch of the Primary Product, EFSA received compositional data for only one batch, whose ID number was not specified. The applicant stated that this batch met the specification parameters and fell within the batch-to-batch variability of the Primary Product. Regarding the water content, the applicant provided data for three replicates, and information on the identity of the underlying batch(es) was lacking. This absence of information on batch-to-batch variability creates a non-standard uncertainty with respect to the reproducibility of the Primary Product (see Section 2.2 of this opinion and Table G.1 of the EFSA guidance document on smoke flavouring (EFSA FAF Panel, 2021)).

**Table 1:** Overview of the compositional data of the Primary Product (Documentation provided to EFSA No. 1 and 2)

| Batch no. | Density (g/L) | Total volatiles (wt%)[(1)] | Identified volatiles (wt%) | Unidentified volatiles (wt%)[(2)] | Total non-volatiles (wt%)[(3)] | Identified non-volatiles (wt%) | Non-Identified non-volatiles (wt%)[(3)] | Water (wt%)[(4)] | Solvent-free fraction (wt %)[(5)] | Ident./quant. proportion of solvent-free fraction (wt%)[(6),(a)] | Ident./quant. proportion of volatile fraction (wt%)[(7),(b)] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| n.a. | 1,073 | ≤ 35.1 | 20.6 | < 14.5 | ≥ 1.6 | – | ≥ 1.6 | 63.3 | 36.7 | 56.1 | ≥ 58.7% |

n.a: not available (i.e. the applicant did not specify the batch number).
(1): Calculated as: 100 wt% – 63.3 wt% (water) – 1.6 wt% (tar).
(2): Calculated as: total volatiles (wt%) – identified volatiles (wt%).
(3): Value corresponds only to the gravimetrically determined tar fraction; no information on further non-volatile constituents available.
(4): Average of three replicates (individual values: 63.7, 62,8, 63.3 wt%; SD (±) 0.5; RSD (%): 0.7).
(5): Average of three replicates (individual values: 36.3, 37.2, 36.7 wt%; SD (±) 0.5; RSD (%): 1.2).
(6): Calculated as: (identified volatiles/solvent-free fraction) × 100.
(7): Calculated as: (identified volatiles/total volatiles) × 100.
(a): Regulatory quality criterion for the applied method according to Regulation (EC) No 627/2006.[6]
(b): Regulatory quality criterion for the applied method according to Regulation (EC) No 627/2006: ≥ 80 (wt%).

[6] Commission Regulation (EC) No 627/2006 of 21 April 2006 implementing Regulation (EC) No 2065/2003 of the European Parliament and of the Council as regards quality criteria for validated analytical methods for sampling, identification and characterisation of primary smoke products. OJ L 109, 22.4.2006, p. 3–6.

The Panel noted the following shortcomings in the data provided by the applicant:

i) The applicant distinguished between volatile and non-volatile constituents on the basis of their boiling points. They referred to Directive 2004/42/EC of the European Parliament and of the Council, which deals with the volatility of substances in paints and varnishes and defines a 'Volatile Organic Compound (VOC)' as any organic compound having an initial boiling point less than or equal to 250°C measured at a standard pressure of 1,013 kPa, and consequently, they considered all constituents eluting in the gas chromatography (GC) chromatogram after catechol (cut-off compound with a GC retention time corresponding to a boiling point of 245°C) as non-volatile constituents. However, this procedure is not in line with the definition of 'volatile fraction' as given in Regulation (EC) No 627/2006 that defines the volatile fraction as 'the part of the solvent free mass, which is volatile and analysable by gas chromatography'. Accordingly, in order to be able to check the performance criteria of the applied methods according to Regulation (EC) No 627/2006, all peaks detected via the applied GC analysis have to be considered as volatile constituents.

ii) The identified volatiles (47 in total) have been quantified by the applicant using appropriate methods and amounted to 20.6 wt% of the Primary Product. However, the unidentified volatile fraction was not adequately quantified. The semi-quantification performed by the applicant on the basis of GC peak areas was not considered appropriate by the Panel.

iii) The applicant isolated a tar fraction from the Primary Product and determined it gravimetrically to be 1.6 wt% (Documentation provided to EFSA No. 1). The Panel agreed that this tar fraction is part of the non-volatile fraction; however, considering that no identifications were performed, the Panel did not agree with the approach of the applicant to include this tar fraction in the fraction of 'identified non-volatiles'. No qualitative/quantitative information on other non-volatile constituents of the Primary Product was provided.

Based on the data available, the non-volatile fraction amounts to at least 1.6 wt%. Accordingly, the amount of total volatiles can be estimated to be not higher than 35.1 wt% (calculated as 100 wt% – 63.3 wt% water – 1.6 wt% tar). Consequently, the identified volatiles constitute at least 58.7 wt% of the volatile fraction. Considering that the Primary Product can be expected to contain non-volatile constituents other than the isolated tar fraction, the volatile fraction is expected to be smaller and the percentage of identified constituents in the volatile fraction probably higher. However, the Panel emphasises that owing to the lack of adequate quantitative data, this value can only be roughly estimated, thus creating a non-standard uncertainty with respect to the chemical composition of the Primary Product (see Section 2.2 of this opinion and Table G.1 of the EFSA guidance document on smoke flavouring [EFSA FAF Panel, 2021]).

*3.1.2.4.1. Chemical characterisation*

The applicant provided data on the content of the major chemical classes in the Primary Product, i.e. acids, carbonyls and phenols (Table 2). The applicant reported that the analyses were performed on 441 production batches (Documentation provided to EFSA No. 1). Water is the solvent of the Primary Product and was determined by the Karl Fischer titration method; data from three replicates were provided.

**Table 2:** Chemical composition of the Primary Product

| | Average (wt%) | SD |
|---|---|---|
| **Acids (wt%) (as acetic acid)** | 10.8[(a)] | 0.28 |
| **Carbonyls (wt%) (as 2-butanone)** | 17.1[(a)] | 2.1 |
| **Phenols (wt%) (as 2,6-dimethoxyphenol)** | 1.64[(a)] | 0.17 |
| **Water (wt%)** | 63.3[(b)] | 0.5 |

SD: standard deviation.
(a): Values are based on analysis of 441 batches.
(b): Values are based on data from three replicates.

Concentrations of arsenic, cadmium, lead and mercury were determined by inductively coupled plasma-mass spectrometry (ICP-MS) and were submitted to EFSA. The analyses were performed on 19 batches of the Primary Product (Table 3) (Documentation provided to EFSA No. 2).

The Panel noted that in all investigated batches, the levels of arsenic and lead were below the limit of quantification (LOQ), i.e. < 0.1 mg/kg and < 0.05 mg/kg for As and Pb, respectively. One batch of the Primary Product contained mercury at the level of LOQ (i.e. 0.005 mg/kg). Cadmium in four batches was below the LOQ of 0.01 mg/kg, in six batches at the level of the LOQ and in nine batches above the LOQ, with the highest measured value being 0.04 mg/kg.

**Table 3:** Toxic elements reported for 19 batches of the Primary Product (Documentation provided to EFSA No. 2)

| | Batch no. (mg/kg) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2152486 | 2152487 | 2152488 | 2152489 | 2152490 | 2152491 | 2152492 | 2152493 | 2152494 | 2152495 | 2152496 | 2152497 | 2152498 | 2152499 | 2152500 | 2152501 | 2152502 | 2152503 | 2152504 | Average (mg/kg) | SD |
| **Arsenic (As)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | – |
| **Cadmium (Cd)** | 0.04 | 0.02 | 0.02 | 0.02 | 0.01 | 0.01 | 0.03 | 0.02 | 0.02 | 0.03 | 0.01 | < 0.01 | < 0.01 | < 0.01 | 0.01 | < 0.01 | 0.01 | 0.02 | 0.01 | < 0.02 | 0.01 |
| **Lead (Pb)** | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | – |
| **Mercury (Hg)** | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | – |

(<): This symbol means that the concentration of the toxic element was below the corresponding LOQ.

*3.1.2.4.2. Identification and quantification of the volatile fraction*

Gas chromatography–mass spectrometry (GC–MS) and gas chromatography–flame ionisation detection (GC–FID) were applied for identification and quantification of the constituents of the volatile fraction of the Primary Product. Individual volatile constituents were considered as identified if their chromatographic (i.e. retention times) and mass spectral data were in agreement with those of reference standards.

The applicant reported that formaldehyde (CAS no.: 50-00-0) and formic acid (CAS no.: 64-18-6) were not detectable through a direct GC–MS analysis, and therefore, they were derivatised with (perfluorophenyl)hydrazine and methanol, respectively. Quantifications were performed by solvent calibration or standard addition. For formaldehyde, an isotopically labelled substance was used as an internal standard for quantification.

Overall, using this approach, 47 constituents were identified and quantified in the Primary Product (Appendix A, Table A.1). The lowest concentration reported by the applicant was $1.61 \times 10^{-4}$ wt% (i.e. 1.6 mg/kg) for 5-hexen-2-one (CAS no.: 109-49-9). The analyses were performed by an external laboratory and the analytical report was submitted to EFSA (Documentation provided to EFSA No. 1). The IDs and the number of the batches subjected to analyses were not specified. Three additional constituents were reported as identified, i.e. (*S*)-2,3-dihydroxypropanal (CAS no.: 497-09-6), 2,4-dimethylphenol (CAS no.: 105-67-9) and 2-propenal (CAS no.: 107-02-8); however, no quantitative data were provided. The 20 principal volatile constituents of the Primary Product are presented in Table 4.

The applicant reported approximately 319 tentatively identified volatile constituents (Documentation provided to EFSA No. 1). EFSA had requested to report the quantitative data on the proportions of (i) identified, (ii) tentatively identified and (iii) unidentified volatile constituents. The applicant replied that the tentatively identified constituents were included as part of the unidentified volatile fraction, however, no additional data were provided in response to this request (Documentation provided to EFSA No. 2). The Panel considered the identification of constituents as tentative when it was (solely) based on structural similarities to identified constituents or when the mass spectral data were only compared to a fragmentation mass spectral library rather than to those of a reference standard. In accordance with the EFSA Scientific Guidance on Smoke Flavourings (EFSA FAF Panel, 2021), the tentatively identified constituents were considered part of the unidentified fraction.

According to the applicant (Documentation provided to EFSA No. 1 and 2), the total volatile fraction of Zesti Smoke Code 10 accounted for 18.5 wt% of the Primary Product. However, the applicant determined the volatile fraction based on the boiling points of the individual constituents, and this is not in line with the definition of 'volatile fraction' as laid down in Regulation (EC) No 627/2006. Therefore, the Panel considered that the total volatile fraction of Zesti Smoke Code 10 was not adequately quantified, and that the fraction of identified and quantified volatiles accounted for approximately 20.6 wt% of the Primary Product. In addition, since the size of the unidentified volatile fraction could only be roughly estimated (see text below Table 1), the Panel could not judge whether the applied methods meet the legal quality criterion that at least 80% by mass of the volatile fraction shall be identified and quantified (Regulation (EC) No 627/2006).

The Panel noted that the current list of identified volatile constituents does not fully match the list of identified volatile constituents provided at the time of the previous EFSA assessment of Zesti Smoke Code 10 (EFSA CEF Panel, 2009). Since no changes were introduced in the manufacturing process, it is likely that the observed differences are mainly due to the fact that in contrast to the previous application, volatiles were only considered as identified if their chromatographic and mass spectrometric data matched those of reference standards. The applicant reported differences in the quantification approaches, such as four different GC columns were employed, derivatisation methods were developed, the quantifications were performed by external standard calibration. It is very likely that the use of more recent analytical techniques allowed the applicant to perform a more accurate characterisation of the volatile fraction. For this reason, the characterisation performed here is more reliable than the characterisation performed in the previous application (EFSA CEF Panel, 2009), and the product evaluated in the present assessment does not fundamentally deviate from the product evaluated in the past.

**Table 4:** Twenty principal volatile constituents of the Primary Product (Documentation provided to EFSA No. 1)

| CAS no. | FL-no | Chemical name[a] | Average concentration (wt%) | |
|---|---|---|---|---|
| | | | Current application[b] | Former application[c] |
| 64-19-7 | 08.002 | Acetic acid | 7.3 | 8.7 |
| 141-46-8 | | Acetaldehyde, hydroxy- | 2.4 | 0.2 |
| 498-07-7 | | β-D-glucopyranose, 1,6-anhydro- | 2.3 | 0.9 |
| 64-18-6 | 08.001 | formic acid | 1.8 | 0.5 |
| 116-09-6 | 07.169 | 1-hydroxypropan-2-one (2-propanone, 1-hydroxy-) | 1.6 | 0.6 |
| 5077-67-8 | 07.090 | 1-hydroxybutan-2-one (1-hydroxy-2-butanone) | 1.0 | 0.1 |
| 67-56-1 | | Methanol | 0.7 | |
| 79-20-9 | 09.023 | Methyl acetate (acetic acid, methyl ester) | 0.6 | |
| 75-07-0 | 05.001 | acetaldehyde | 0.4 | |
| 497-23-4 | former 10.066[d] | furan-2(5*H*)-one (2(5*H*)-furanone) | 0.3 | 0.1 |
| 120-80-9 | 04.029 | benzene-1,2-diol (catechol) | 0.3 | |
| 80-71-7 | 07.056[e] | 3-methylcyclopentan-1,2-dione (2-cyclopenten-1-one, 2-hydroxy-3-methyl-) | 0.3 | 0.4 |
| 91-10-1 | 04.036 | 2,6-dimethoxyphenol (phenol, 2,6-dimethoxy-) | 0.2 | 0.4 |
| 98-01-1 | 13.018 | Furfural | 0.2 | 0.1 |
| 50-00-0 | | Formaldehyde | 0.2 | |
| 67-64-1 | 07.050 | Acetone | 0.1 | |
| 4451-30-3 | | 1,4:3,6-dianhydro-α-D-glucopyranose | 0.1 | 0.08 |
| 431-03-8 | 07.052 | diacetyl (2,3-butanedione) | 0.08 | |
| 90-05-1 | 04.005 | 2-methoxyphenol (phenol, 2-methoxy-) | 0.07 | 0.2 |
| 108-95-2 | 04.041 | phenol | 0.06 | 0.3 |

CAS: Chemical Abstract Service; FL-no: FLAVIS number; wt: weight.

(a): In case a constituent of the Primary Product is an authorised flavouring substance (FL-no), the assigned chemical name corresponds to the respective entry in the EU Union List of flavourings. Deviating chemical names reported by the applicant in the dossier are given in brackets, if applicable.

(b): The values reported are claimed to be obtained from a duplicate analysis, however, the individual values nor the batch IDs were provided.

(c): From the data presented in the previous safety evaluation of the Primary Product (EFSA CEF Panel, 2009).

(d): 'Former FL-number' refers to substances that were initially included in the evaluation programme but were not included or were removed/withdrawn from the Union List.

(e): [FL-no: 07.056] refers to the mixture of the tautomeric forms of 3-methylcyclopentan-1,2-dione.

#### *3.1.2.4.3. Characterisation of the non-volatile fraction*

The applicant isolated a tar fraction from the Primary Product; the residue remaining after mixing the Primary Product with water and subsequent centrifugation amounted to 1.6 wt%. The Panel considered that in contrast to the procedure followed by the applicant, this gravimetrically determined tar fraction cannot be included in the list of identified non-volatiles. The Panel considered this tar fraction to be part of the unidentified non-volatiles.

As discussed in Section 3.1.2.4, the applicant distinguished between volatile and non-volatile constituents on the basis of their boiling points and consequently considered all constituents eluting in the GC chromatogram after catechol (cut-off compound with a GC retention time corresponding to a boiling point of 245°C) as non-volatile constituents. However, this procedure is not in line with the definition of 'volatile fraction' as given in Regulation (EC) No 627/2006 that defines the volatile fraction as 'the part of the solvent free mass, which is volatile and analysable by gas chromatography'.

Therefore, the Panel considered none of the constituents detected by the applicant via the applied GC analyses as non-volatile.

Accordingly, apart from the gravimetrically determined amount of the water-insoluble tar fraction, no further information on other non-volatile constituents of the Primary Product has been provided by the applicant.

*3.1.2.4.4. Unidentified fraction*

The Panel concluded that based on the compositional data provided by the applicant, 63.3 wt% of water and 20.6 wt% of volatile constituents can be considered as identified fractions of the Primary Product. Accordingly, the unidentified fraction of the Primary Product amounts to 16.1 wt%.

*3.1.2.4.5. Overall composition of the Primary Product*

Based on the chemical data provided and in light of the shortcomings outlined above, the overall composition of Zesti Smoke Code 10 (wt% of Primary Product) and the composition (wt%) of the solvent-free fraction, as assessed by the Panel, are shown in Figures 1 and 2, respectively.

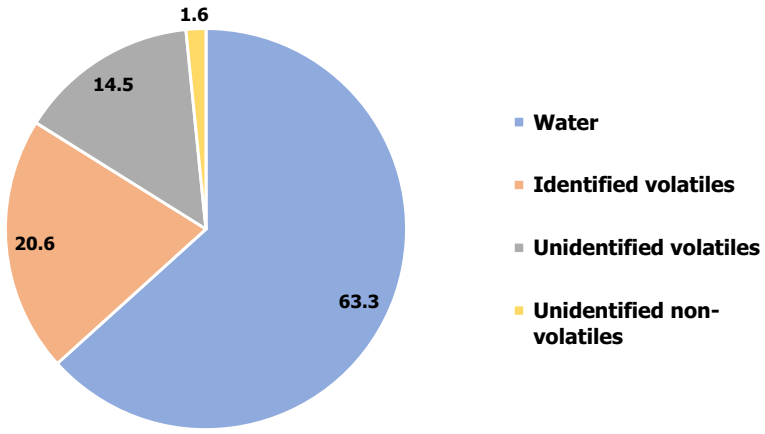


**Figure 1:** Overall composition of Zesti Smoke Code 10 (wt% of Primary Product), as assessed by the Panel. In the pie chart the symbols '<' and '>' as shown in Table 1 are not taken into account

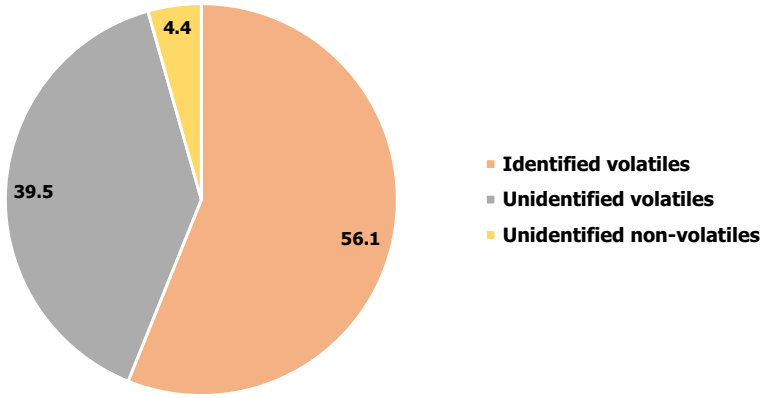


**Figure 2:** Composition (wt%) of the solvent-free fraction of Zesti Smoke Code 10, as assessed by the Panel. In the pie chart the symbols '<' and '>' as shown in Table 1 are not taken into account

The applicant claimed that the analyses on the volatile and non-volatile fractions were performed in duplicate. However, since the individual values and the batch IDs were not provided, the Panel could not verify this information. This creates a non-standard uncertainty with respect to the reproducibility of the Primary Product (see Section 2.2 of this opinion and Table G.1 of the EFSA guidance document on smoke flavouring (EFSA FAF Panel, 2021)).

Despite the limitations outlined above, the Panel anticipates that for the investigated batch(es) of the Primary Product, the identified and quantified proportion of the solvent-free fraction is higher than

50%, thus meeting the legal quality criterion for the applied methods, i.e. at least 50% by mass (wt %) of the solvent-free fraction shall be identified and quantified (Regulation (EC) No 627/2006).

However, given the shortcomings of the quantification approach employed by the applicant (see Section 3.1.2.4), the Panel could not judge whether the applied methods meet the legal quality criterion that at least 80% of the volatile fraction shall be identified and quantified (Regulation (EC) No 627/2006). This creates a non-standard uncertainty with respect to the chemical composition of the Primary Product (see Section 2.2 of this opinion and Table G.1 of the EFSA guidance document on smoke flavouring (EFSA FAF Panel, 2021)).

### 3.1.2.5. Polycyclic aromatic hydrocarbons (PAHs)

Analytical data on the contents of 16 PAHs were provided for 19 batches of the Primary Product (Table 5). The analysis meets the performance criteria as set in Regulation (EC) No 627/2006. The levels reported for the individual PAHs (Table 5) are consistently below the minimum required limits of quantification according to Regulation (EC) No 627/2006.

The levels of benzo[a]pyrene and benzo[a]anthracene are below their respective limits of 10 and 20 μg/kg as laid down in the Regulation (EC) No 2065/2003.

**Table 5:** Concentrations of PAHs in the Primary Product from 19 batches (for batch numbers see Table 3) (Documentation provided to EFSA No. 1). Averages and standard deviations were not included, as all the PAHs were reported at concentrations below their respective LOQs

| PAH | Conc. range (μg/kg) |
|---|---|
| **benzo[a]anthracene**[(a)] | < 0.5[(b)] |
| **chrysene**[(a)] | < 0.5[(b)] |
| **benzo[b]fluoranthene**[(a)] | < 0.5[(b)] |
| benzo[k]fluoranthene | < 0.5[(b)] |
| benzo[j]fluoranthene | < 0.5[(b)] |
| **benzo[a]pyrene**[(a)] | < 0.5[(b)] |
| indeno[123-cd]pyrene | < 0.5[(b)] |
| dibenzo[a,h]anthracene | < 0.5[(b)] |
| benzo[g,h,i]perylene | < 0.5[(b)] |
| dibenzo[a,l]pyrene | < 1.0[(b)] |
| dibenzo[a,i]pyrene | < 1.0[(b)] |
| dibenzo[a,h]pyrene | < 1.0[(b)] |
| dibenzo[a,e]pyrene | < 1.0[(b)] |
| cyclopenta[cd]pyrene | < 1.0[(b)] |
| 5-methylchrysene | < 1.0[(b)] |
| benzo[c]fluorene | < 1.0[(b)] |
| **PAH4** | < 2.0[(c)] |

PAH: polycyclic aromatic hydrocarbon.
(a): PAHs printed in bold are used for the evaluation of the exposure to these contaminants (see Section 3.3.3.2).
(b): Value below the corresponding limit of quantification (LOQ).
(c): Value below the sum of the LOQs for PAH4 in the individual batches.

### 3.1.2.6. Batch-to-batch variability

The batch-to-batch variability was investigated in 441 batches with production dates spanning from years 2020 to 2021. The applicant informed that the batches were chosen '*without modification or selection criteria bias for optimal conditions and representative of the range of conditions that are used in the pyrolysis step*' (Documentation provided to EFSA No. 1).

The monitored parameters were pH, staining index, specific gravity and the contents of total acids, carbonyls, phenols and hydroxyacetaldehyde (Table 6). The analytical methods were described, and the reports were submitted to EFSA (Documentation provided to EFSA No. 1). Data for the individual batches were not provided. Based on the reported standard deviations, the Panel considered that the batch-to-batch variability of the investigated 441 batches was acceptable for the parameters monitored.

**Table 6:** Batch-to-batch variability of the Primary Product

| | Average | SD | RSD (%) |
|---|---|---|---|
| **pH** | 2.2 | 0.1 | 4.5 |
| **Staining index** | 90.0 | 11.1 | 12.3 |
| **Specific gravity** | 1.085 | 0.005 | 0.5 |
| **Total acidity (wt%)** | 10.8 | 0.3 | 2.8 |
| **Carbonyls (wt%)** | 17.1 | 2.1 | 12.3 |
| **Phenols (wt%)** | 1.64 | 0.17 | 10.4 |
| **Hydroxyacetaldehyde (%)** | 2.4 | 0.4 | 16.7 |

wt: weight; SD: standard deviation; RSD: relative standard deviation.

In addition, the applicant performed statistical analyses to assess the variability, i.e. means and variances, of 18 volatile constituents in 26 batches of the Primary Product randomly selected from July 2020 to January 2022 (Documentation provided to EFSA No. 1). The report submitted to EFSA is based on the examination of GC-FID data from quality control analyses (Documentation provided to EFSA No. 1). The 18 peaks subjected to statistical analysis covered 62% of the total GC peak area. Based on the reported relative standard deviation, on average < 16% for the individual peaks, the Panel considered the batch-to-batch variability of the investigated batches as acceptable.

#### 3.1.2.7. Solubility and particle size

Water solubility and particle size of the Primary Product were not determined by the applicant in the original technical dossier (Documentation provided to EFSA No. 1). In addition, the applicant states in the dossier that '*Generally, the product is clear and with minimal precipitate in the final product*.' This suggests that the Primary Product might contain small particles. Therefore, the applicant was requested to investigate the potential presence of small particles including nanoparticles in the Primary Product, in line with the EFSA 'Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles' (EFSA Scientific Committee, 2021a). The applicant replied that the Primary Product, being a complex mixture, could not be assessed according to the above-mentioned guidance and/or the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 105 (OECD, 1995) method. Instead, the applicant used published data (or the predictive programme EPIWIN when published data were not available) to report the water solubilities of the identified constituents of the Primary Product (Documentation provided to EFSA No. 3). Specifically, the applicant used a mathematical approach according to which the concentration of each identified constituent in the Primary Product was below its maximum solubility in water, thus they were all claimed to be soluble at their levels in the Primary Product. Hence, the applicant concluded that the Primary Product did not require additional analytical testing to clarify its water solubility and/or particle size distribution (Documentation provided to EFSA No. 3).

The Panel noted that the Primary Product contains 16.1 wt% of unidentified matter (see Section 3.1.2.4.4). Since this uncharacterised fraction was not considered by the applicant, the potential presence of small particles including nanoparticles cannot be excluded. This creates a non-standard uncertainty with respect to the solubility and particle size of the Primary Product (see Section 2.2 of this opinion and Table G.1 of the EFSA guidance document on smoke flavouring (EFSA FAF Panel, 2021)).

### 3.1.3. Specifications

The applicant provided the required product specification data and reported that the Primary Product Zesti Smoke Code 10 is manufactured within its proposed specifications (Documentation provided to EFSA No. 1 and 2). Information on parameters relevant for the specifications has been compiled by the Panel in Table 7.

**Table 7:** Relevant information for specifications of the Primary Product

| | Specifications for Zesti Smoke Code 10 as proposed by the applicant | Specifications as reported in (EFSA CEF Panel, 2009) | Specifications as laid down in Regulation (EU) No 1321/2013 |
|---|---|---|---|
| **Description** | n.a. | n.a. | n.a. |
| **Source material** | | | |
| • Woods | 40–50% of white oak (*Quercus Alba*) and 50–60% of hickory (*Carya Ovata*) | n.a. | 50–60% of hickory (*Carya ovata*), 40–50% oak (*Quercus* alba) |
| **Identity parameters** | | | |
| • Physico-chemical parameters | | | |
| – pH | 2.0–2.5 | 2.0–2.5 | 2.0–2.5 |
| – density | 1.070–1.088 (g/mL) | 1.070–1.088 (as specific gravity, at 25°C) | n.a. |
| – refraction index | 28–33 (as °BRIX) | n.a. | n.a. |
| – staining index | 70–100 | 70–100 | n.a. |
| **Chemical composition:** | | | |
| • Chemical classes: | | | |
| – Acids | 10.5–11.0 wt% | 10.5–11.0% (as acetic acid) | 10.5–11.0% (as acetic acid) |
| – Carbonyls | 15.0–25.0 wt% | 15–25 g/100 mL | 15–25 g/100 mL |
| – Phenols | 1.2–2.2 wt% | 12–22 mg/mL | 12–22 mg/mL |
| – Water | 62.3–65.7 wt% | n.a. | 62.3–65.7% |
| 20 principal constituents of the volatile fraction | see Table 4 | n.a. | |
| **Purity** | | | |
| – benzo[a]pyrene | < 0.5 µg/kg | n.a. | n.a. |
| – benzo[a] anthracene | < 0.5 µg/kg | n.a. | n.a. |
| – PAH4[(1)] | < 2.0 µg/kg | n.a. | n.a. |
| • Toxic elements | | | n.a. |
| – Lead | < 0.05 mg/kg | n.a. | < 5.0 mg/kg |
| – Arsenic | < 0.1 mg/kg | n.a. | < 3.0 mg/kg |
| – Cadmium | 0.02 mg/kg | n.a. | < 1.0 mg/kg |
| – Mercury | 0.005 mg/kg | n.a. | < 1.0 mg/kg |

wt: weight; n.a.: not available.
(1): This value was calculated by the Panel, considering the specification limits proposed by the applicant, for the individual PAH4, i.e. benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene (Documentation provided to EFSA No. 1).

The Panel noted that the analytical data for the batches analysed indicate that actual concentrations of toxic elements, reported in Table 3, are lower than the limits laid down in Regulation (EU) No 1321/2013. The Panel further noted that for cadmium, the applicant proposed specification limits that are lower than the highest measured value of 0.04 mg/kg (see Table 3).

For the PAHs, the Panel noted that the analytical data indicated that their actual concentrations (Table 5) are lower than the limits laid down in Regulation (EC) No 2065/2003 for benzo[a]pyrene and

benzo[a]anthracene. The Panel further noted that the specification limit for PAH4 corresponds to the sum of the LOQs (i.e. < 2.0 μg/kg; see Table 5) of the four individual PAHs in the Primary Product.

### 3.1.4. Stability and fate in food

The applicant informed that stability and shelf-life tests are performed regularly on randomly selected batches of the Primary Product to verify that the production batches comply with the internal 2-years product specifications. The batches are assessed for the parameters of total acids, phenols, carbonyls, °BRIX, pH, hydroxyacetaldehyde and specific gravity. During storage (in light-controlled containers at ambient temperature), the production batches are tested at certain time thresholds, generally, at months 1, 2, 3, 6, 9, 12, 18 and 24. The data provided by the applicant are shown in Table 8. Information on the number of batches monitored for stability and their ID numbers not were not provided. Based on the average values, all parameters studied remained within their specification ranges over the 2 years storage period, although some batches fell below the specification limit values for total phenols and total carbonyls. Based on the average values, the concentrations of phenols and carbonyls showed significant decreases; information on possible degradation/reaction products was not provided.

In contrast to the requirements outlined in the EFSA guidance document on smoke flavourings (EFSA FAF Panel, 2021), the applicant did not provide stability data based on the analysis of individual volatile constituents. These data gaps create a non-standard uncertainty with respect to the stability of the Primary Product (see Section 2.2 of this opinion and Table G.1 of the EFSA guidance document on smoke flavouring (EFSA FAF Panel, 2021)).

No analytical data on the stability of the Primary Product in commercial formulations or in the proposed food categories were provided.

**Table 8:** Parameters and (2-years) specification range values used to monitor the storage stability and shelf-life of the Primary Product, as provided by the applicant

| | Spec. at time of manufacture | 2 years range | Average at 2 years | % change at 2 years | SD | RSD (%) |
|---|---|---|---|---|---|---|
| **Total acids (wt%)** | 10.5–11.0 | 10.5–11.0 | 10.8 | 1.6% | 0.2 | 1.9 |
| **Phenols (wt%)** | 1.2–2.2 | 0.78–2.2 | 1.6 | −32.0% | 0.01 | 5.8 |
| **Carbonyls (wt%)** | 15–25 | 12.0–25.0 | 17.1 | −27.1% | 1.5 | 8.8 |
| **°BRIX** | 28–33 | 28–33 | 31.0 | −7.7% | 0.3 | 1.0 |
| **pH** | 2.0–2.5 | 2–2.5 | 2.2 | −7.3% | 0.1 | 4.5 |
| **Hydroxyacetaldehyde (%)** | 1.5–3.0 | 1.5–3.0 | 2.4 | −4.0% | 0.2 | 8.3 |
| **Specific gravity** | 1.070–1.088 | 1.070–1.088 | 1.085 | −1.0% | 0.002 | 0.2 |

wt: weight; SD: standard deviation; RSD: relative standard deviation.

## 3.2. Proposed use and use levels

The applicant applied for a renewal of authorisation of the Primary Product Zesti Smoke Code 10 for use in foods at the proposed maximum and expected typical use levels as presented in Table 9.

The proposed maximum and expected typical use levels were used to assess the dietary exposure to this Primary Product (see Section 3.3.2).

**Table 9:** Proposed maximum and expected typical use levels of the Primary Product (mg/kg) in food categories according to Annex II of Regulation (EC) No 1333/2008[7]

| Food category number | Food category Name | Restrictions/exceptions | Proposed maximum use levels (mg/kg)[(a)] | Expected typical use levels (mg/kg)[(a)] |
|---|---|---|---|---|
| 1.7.2 | Ripened Cheese | Only in 'Cheese, camembert' and 'Cheese, edam' and 'Cheese, gouda' and 'Cheese, maasdam' and 'Cheese, morbier' and 'Cheese, provolone' and 'Cheese, raclette' and 'Cheese, scamorza' and 'Cheese, smoked gouda' and 'Cheese, cheddar' and 'Cheese, emmental' and 'Cheese, caciocavallo' and 'Cheese, pecorino romano' | 1,000 | 500 |
| 1.8 | Dairy analogues, including beverage whiteners | Only in 'Tofu' and 'Imitation cheese' | 500 | 200 |
| 8.2 | Meat preparations as defined by Regulation (EC) No 853/2004 | Only in 'Marinated meat' and 'Chipolata-type sausage' | 1,000 | 500 |
| 8.3 | Meat Products | Only in 'Cured pork fat' and 'Canned meat' and 'Cured seasoned poultry meat' and 'Cooked cured (or seasoned) bovine meat' and 'Cooked cured (or seasoned) poultry meat' and 'Italian-style sausage' and 'Meat spread' and 'Other cured meat' and 'Ham, pork' and 'Tiroler speck' and 'Bacon' and 'Pancetta' and 'Ham, beef' and 'Cooked pork ham' and 'Pastrami, pork' and 'Pastrami, lamb' and 'Thuringian sausage' and 'Mettwurst type sausage' and 'Teewurst-type sausage' and 'Salami-type sausage' and 'Knackwurst-type sausage' and 'Liver-type sausage' and 'Polish-type cooked sausage' and 'Mortadella-type sausage' and 'Bologna-type sausage' and 'Blood-type sausage' and 'Chorizo and similar' and 'Linguica, sausage' and 'Snack sausages (like Cabanos and landjäger)' and 'Ripened kolbasz' and 'Cooked salami' and 'Frankfurter sausage' and 'Wiener sausage' and 'Beerwurst' and 'Bockwurst' and 'Cervelat (swiss type)' | 2,500 | 1,000 |
| 9.2 | Processed fish and fishery products including molluscs and crustaceans | Only in 'Smoked seafood' and 'Marinated/ pickled fish' and 'Smoked salmon' and 'Smoked herring' and 'Other smoked fishes' and 'Canned salmon' and 'Canned mackerel' and 'Canned herring' and 'Canned sprats' and 'Canned sardines' and 'Smoked mackerel' | 2,000 | 700 |
| 9.3 | Fish roe | Only in 'Fish roe'[(b)] | 2,000 | 700 |
| 12.2 | Herbs, spices, seasonings | Only in 'Seasonings and extracts' and 'Coriander seed' and 'Black pepper' and 'Paprika powder' | 3,000 | 1,000 |
| 12.4 | Mustard | Only in 'Mustard and related sauces'[(b)] | 100 | 30 |

[7] 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, p. 16–33.

| Food category number | Food category Name | Restrictions/exceptions | Proposed maximum use levels (mg/kg)[(a)] | Expected typical use levels (mg/kg)[(a)] |
|---|---|---|---|---|
| 12.5 | Soups and broths | Only in 'Potato soup' and 'Legume (beans) soup' and 'Mushroom soup' and 'Cereal products and grains-based soup' and 'Stock cubes or granulate, vegetables' and 'Grains soup, dry' and 'Meat and vegetable soup, dry' and 'mixed vegetables soup, dry' and 'Potato soup, dry' and 'Stock cubes or granulate, fish' and 'Mixed vegetables soup' and 'Stock cubes or granulate, beef' and 'Meat soup, with pieces' and 'Meat and vegetable soup, with puree or pieces' and 'Meat and vegetable soup, clear' | 3,00 | 100 |
| 12.6 | Sauces | Only in 'Barbecue or steak sauces' | 3,000 | 2,000 |
| | | Only in 'Tomato ketchup and related sauces' | 100 | 30 |
| 12.9 | Protein products, excluding products covered in category 1.8 | Only in 'Meat imitates' | 1,000 | 500 |
| 15.1 | Potato-, cereal-, flour- or starch-based snacks | Only in 'Snacks other than chips and similar' and 'Corn chips' and 'Tortilla chips' and 'Corn curls' and 'Rice chips' and 'Potato crisps or sticks' | 2,000 | 700 |
| 15.2 | Processed nuts | Only in 'Almonds' and 'Peanuts' | 2,000 | 700 |

(a): Use levels are provided for the foods as consumed.
(b): As provided by the applicant. Since this food category is not further divided into sub-categories, this restriction has no impact on the DietEx exposure estimates.

## 3.3. Exposure

### 3.3.1. Food consumption data used for exposure assessment

The food consumption data used for the exposure assessment are from the EFSA Comprehensive European Food Consumption Database Comprehensive database.[8] This database contains food consumption data at the level of the individual consumer from the most recent national dietary surveys carried out in EU countries and includes the currently best available food consumption data across the EU. These data cover infants (from 0 weeks of age), toddlers (1–2 years), children (3–9 years), adolescents (10–17 years), adults (18–64 years) and the elderly (65 years and older). As these data were collected by different methodologies, direct country-to-country comparisons of exposure estimates based on these data may not be appropriate.

The dietary exposure to the Primary Product was calculated by the applicant and EFSA using Food Additive Intake Model (FAIM, version 2.1) and DietEx tools. The food consumption data in both tools are based on the version of the Comprehensive Database that was published in July 2021. These data cover 42 dietary surveys carried out in 22 EU countries (Table 10).

[8] https://www.efsa.europa.eu/en/data-report/food-consumption-data

**Table 10:** Population groups and countries considered for the exposure estimates of the Primary Product with FAIM and DietEx[9]

| Population | Age range | Countries with food consumption surveys covering more than 1 day |
|---|---|---|
| Infants | From 0-12 weeks[(a)] up to and including 11 months of age | Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain |
| Toddlers[(b)] | From 12 months up to and including 35 months of age | Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, the Netherlands, Portugal, Slovenia, Spain |
| Children[(c)] | From 36 months up to and including 9 years of age | Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, the Netherlands, Portugal, Spain, Sweden |
| Adolescents | From 10 years up to and including 17 years of age | Austria, Belgium, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden |
| Adults | From 18 years up to and including 64 years of age | Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden |
| The elderly[(c)] | From 65 years of age and older | Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden |

(a): FAIM includes infants from 12 weeks of age and DietEx infants from 0 weeks of age.
(b): The term 'toddlers' in the Comprehensive Database (EFSA, 2011) corresponds to 'young children' (from 12 months up to and including 35 months of age) in Regulations (EC) No 1333/2008 and (EU) No 609/2013[9].
(c): In FAIM, the terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Comprehensive Database (EFSA, 2011).

The food consumption data from the Comprehensive Database in FAIM are codified according to the food categories as presented in Annex II, Part D, of Regulation (EC) No 1333/2008, which is the relevant regulation for the food categories of the smoke flavourings. In DietEx, these consumption records are codified according to the FoodEx2 food classification and description system. As FoodEx2 includes more information on the foods coded in the food consumption data, this tool will potentially result in less conservative estimates of dietary exposure compared to FAIM.

### 3.3.2. Exposure assessment of the Primary Product

Using both FAIM and DietEx, dietary exposure to the Primary Product was calculated by multiplying the relevant use level for each food category or FoodEx2 code with its respective consumption amount for each individual. This was done for all individuals in the surveys (i.e. the estimates are not based on consumers only). The exposures per food category or FoodEx2 code were subsequently added and divided by the individual body weight (bw) (as registered in the consumption survey) to derive an individual total exposure per day expressed per kilogram bw. These exposure estimates were averaged over the number of survey days in the survey, resulting in an individual average exposure per day. Dietary surveys with only 1 day per subject were excluded as they are not considered adequate to assess repeated exposure. The calculations resulted in distributions of individual exposure per survey and population group. Based on these distributions, the mean and the 95th percentile of exposure were calculated per survey and population group. The 95th percentile of exposure was only calculated for those population groups with a sufficiently large sample size to obtain a reliable estimate (EFSA, 2011).

[9] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–54.

In FAIM, the infant population considers infants from 12 weeks up to and including 11 months of age. In DietEx, the infant population considers infants from 0 weeks up to and including 11 months of age.

#### 3.3.2.1. Exposure assessment using FAIM

The applicant provided estimates of dietary exposure to the Primary Product using FAIM, based on the proposed maximum (proposed maximum use level exposure assessment scenario) and expected typical use levels (expected typical use level exposure assessment scenario) (Documentation provided to EFSA No.1). These estimates were re-calculated by EFSA.

In FAIM, use levels were linked to the corresponding food categories according to the instructions provided for its use.[10] Furthermore, all foods belonging to the food categories (FC) were included in the assessment without applying the restrictions/exceptions as indicated in Table 9. This tool does not allow to include or exclude specific foods from the exposure assessment. See Annex A1 for the food categories and use levels considered in FAIM.

#### Exposure estimates using FAIM

In Table 11, the dietary exposure estimates of the Primary Product with FAIM are presented.

**Table 11:** Summary of dietary exposure to the Primary Product from its proposed maximum and expected typical use levels as a smoke flavouring in six population groups, estimated with FAIM (minimum-maximum across the dietary surveys in mg/kg body weight (bw) per day)

| | Infants (12 weeks-11 months) (n = 11/9) | Toddlers (12–35 months) (n = 15/13) | Children (3–9 years) (n = 19/19) | Adolescents (10–17 years) (n = 21/20) | Adults (18–64 years) (n = 22/22) | The elderly (≥ 65 years) (n = 22/21) |
|---|---|---|---|---|---|---|
| **Proposed maximum use level exposure assessment scenario** | | | | | | |
| Mean | 0.2–7.6 | 2.7–14.7 | 3.2–16.3 | 2.5–9.4 | 1.6–6.3 | 1.0–4.6 |
| 95th percentile | 0.5–24.0 | 10.2–49.6 | 8.8–44.8 | 5.2–25.4 | 3.9–18.0 | 2.8–12.8 |
| **Expected typical use level exposure assessment scenario** | | | | | | |
| Mean | 0.1–3.0 | 1.1–6.2 | 1.3–6.7 | 1.0–3.9 | 0.7–2.7 | 0.4–1.9 |
| 95th percentile | 0.2–9.9 | 3.8–18.6 | 3.6–16.5 | 2.3–10.0 | 1.6–7.2 | 1.2–4.9 |

n: number of surveys for which a mean/P95 could be calculated.

At the proposed maximum use levels, the mean exposure to the Primary Product from its use as a smoke flavouring ranged from 0.2 mg/kg bw per day in infants to 16.3 mg/kg bw per day in children. The 95th percentile of exposure to the Primary product ranged from 0.5 mg/kg bw per day in infants to 49.6 mg/kg bw per day in toddlers.

At the expected typical use levels, the mean exposure ranged from 0.1 mg/kg bw per day in infants to 6.7 mg/kg bw per day in children, and the 95th percentile of exposure from 0.2 mg/kg bw per day in infants to 18.6 mg/kg bw per day in toddlers.

The Primary Product is requested for renewal of authorisation in 13 food categories (Table 9). For all these 13 food categories, it was assumed that 100% of the foods belonging to these food categories will contain the Primary Product at the proposed maximum or expected typical use levels. As it is unlikely that the product will be added to all foods and given the restrictions/exceptions for most of the food categories (Table 9), the Panel considered that the calculated exposure to the Primary Product using FAIM is an overestimation of the expected exposure in EU countries if this Primary Product is used at the proposed maximum or expected typical use levels.

Additionally, overall sources of standard uncertainties (Annex A9) also contributed to an overestimation of the exposure.

Detailed results per population group and survey are presented in Annexes A2 (proposed maximum use level exposure assessment scenario) and A3 (expected typical use level exposure assessment scenario).

[10] Link to FAIM instructions: https://www.efsa.europa.eu/sites/default/files/applications/FAIM-instructions.pdf

### 3.3.2.2. Exposure assessment using DietEx

The applicant provided also estimates of dietary exposure to the Primary Product using DietEx, based on the proposed maximum and expected typical use levels (Documentation provided to EFSA No.1). These estimates were re-calculated by EFSA, following a submission of updated uses and use levels from the applicant (Documentation provided to EFSA n.2).

To assess the exposure using DietEx, the applicant provided a list of FoodEx2 codes per food category (Documentation provided to EFSA No.2). Using FoodEx2 codes, the applicant selected the foods to which the Primary Product could be added per food category, considering the restrictions/ exceptions (Table 9). For some foods belonging to FC 12.5 (Soups and broths), the consumption in DietEx is reported in the dry form (not as consumed) whereas the use levels refer to foods as consumed. These foods include 'stock cubes or granulate, fish', 'stock cubes or granulate, vegetables', 'stock cubes or granulate, beef', 'grains soup, dry', 'potato soup, dry', 'meat and vegetables soup, dry' and 'mixed vegetables soup, dry'. To assess the exposure to the Primary Product via these foods, a factor was applied to the use levels to convert them to levels in the dry form of the food. These factors were derived from EFSA report on dilution factors (EFSA, 2018). For the exposure assessment using FAIM, these factors were not used, because it is not possible to distinguish between consumption levels of foods 'as consumed' or 'in the dry form' in FAIM. This may have resulted in an underestimation of the exposure to the Primary Product via FC 12.5 using FAIM.

See Annex A4 for the list of FoodEx2 codes per food category (according to Annex II of Regulation (EC) No 1333/2008), use levels and factors that were used in the exposure assessment using DietEx.

#### Exposure estimates using DietEx

In Table 12, the dietary exposure estimates of the Primary Product with DietEx are presented.

**Table 12:** Summary of dietary exposure to the Primary Product from its proposed maximum and expected typical use levels as a smoke flavouring in six population groups, estimated with DietEx (minimum–maximum across the dietary surveys in mg/kg body weight (bw) per day)

| | Infants (0 weeks-11 months) (n = 12/11)[a] | Toddlers (12–35 months) (n = 15/13) | Children (3–9 years) (n = 19/19) | Adolescents (10–17 years) (n = 21/20) | Adults (18–64 years) (n = 22/22) | The elderly (≥ 65 years) (n = 33/29)[b] |
|---|---|---|---|---|---|---|
| **Proposed maximum use level exposure assessment scenario** | | | | | | |
| Mean | 0.02–1.2 | 0.4–4.6 | 0.7–4.2 | 0.5–2.1 | 0.5–1.6 | 0.2–1.3 |
| 95th percentile | None–6.1 | 2.0–13.0 | 2.8–12.4 | 2.5–6.0 | 1.6–5.3 | 0.8–4.1 |
| **Expected typical use level exposure assessment scenario** | | | | | | |
| Mean | 0.01–0.5 | 0.1–1.8 | 0.3–1.6 | 0.2–0.8 | 0.2–0.6 | 0.1–0.5 |
| 95th percentile | None–2.4 | 0.7–5.2 | 1.0–4.7 | 0.9–2.3 | 0.6–2.0 | 0.3–1.6 |

n: number of surveys for which a mean/P95 could be calculated.

(a): The number of surveys for infants is different from FAIM as the age range for this population group differs between the two tools.

(b): DietEx provides exposure estimates for the elderly and the very elderly population groups. To ease the reading, and for consistency with FAIM, exposure results were reported as the range of these two population groups (i.e. the min being the minimum between both populations and max being the maximum between both populations).

At the proposed maximum use levels, the mean exposure to the Primary Product from its use as a smoke flavouring ranged from 0.02 mg/kg bw per day in infants to 4.6 mg/kg bw per day in toddlers. The 95th percentile of exposure to the Primary Product ranged from no dietary exposure in infants to 13.0 mg/kg bw per day in toddlers.

At the expected typical use levels, the mean exposure ranged from 0.01 mg/kg bw per day in infants to 1.8 mg/kg bw per day in toddlers, and the 95th percentile of exposure from no dietary exposure in infants to 5.2 mg/kg bw per day in toddlers.

As for FAIM, the Panel considered that the calculated exposure to the Primary Product using DietEx is an overestimation of the expected exposure in EU countries at the proposed maximum or expected

typical use levels. In fact, it is assumed that the Primary Product is used in all foods within food categories without restrictions/exceptions, as well as in all foods within a food category with restrictions/exceptions that meet these restrictions/exceptions.

Additionally, overall sources of standard uncertainties (Annex A9) also contributed to an overestimation of the exposure.

Detailed results per population group and survey are presented in Annexes A5 (proposed maximum use level exposure assessment scenario) and A6 (expected typical use level exposure assessment scenario).

**Main FoodEx2 codes contributing to exposure to the Primary Product using DietEx**

Under the conservative assumptions mentioned above, the main FoodEx2 codes contributing to the total mean exposure to the Primary Product for both exposure scenarios contributing to at least 30% to the total mean exposure in at least one population group in one survey, listed in order of the number of the FCs, are:

- Cheese, gouda belonging to FC 01.7.2.
- Salami-type sausage belonging to FC 08.3.1.
- Cooked pork ham belonging to FC 08.3.2.
- Ham, pork belonging to FC 08.3.2.
- Frankfurter sausage belonging to FC 08.3.2.
- Wiener sausage belonging to FC 08.3.2.
- Cooked cured (or seasoned) poultry meat belonging to FC 08.3.2.
- Potato crisps or sticks belonging to FC 15.1.

Considering the conservative nature of the underlying assumption that 100% of the foods within the FoodEx2 codes (with the restrictions/exceptions, Table 9) contain the Primary Product, the Panel emphasises that the FoodEx2 codes listed here may not reflect the FoodEx2 codes that contribute most to the exposure in real life.

Detailed results of the contributing FoodEx2 codes per population group and dietary survey are presented in Annex A (Annexes A7 and A8).

#### 3.3.2.3. Comparison FAIM versus DietEx

The Primary Product is requested to be authorised in 13 food categories, most having restrictions/ exceptions. Using FAIM, it was assumed that 100% of the foods belonging to these food categories will contain the Primary Product at the proposed maximum or expected typical use levels. Using DietEx, the restrictions/exceptions of use were considered by identifying foods via FoodEx2 codes to which the Primary Product may be added. In the exposure assessment, all foods belonging to a FoodEx2 code were assumed to contain the Primary Product at the proposed maximum or expected typical use levels. For both tools, the assumption of 100% use led to an overestimation of the exposure, together with the sources of standard uncertainties (described in Annex A9). However, this overestimation is expected to be less pronounced (i.e. less conservative) in DietEx than in FAIM as DietEx allows the selection of foods within food categories to which the Primary Product may be added. Therefore, the DietEx exposure estimates will be used for the risk assessment, because in general these estimates are considered more refined than the FAIM exposure estimates.

### 3.3.3. Anticipated exposure to impurities in the Primary Product

The potential exposure to impurities arsenic, lead, cadmium, mercury and PAHs (as PAH4) from the use of the Primary Product can be calculated by assuming that they are present in the Primary Product up to a limit value and then by calculating pro-rata to the estimates of exposure to the Primary Product itself.

With regard to the dietary exposure to the Primary Product, the Panel considered the highest mean and the highest 95th percentile exposure estimates resulting from the exposure assessment using DietEx among the different population groups, i.e. 4.6 mg/kg bw per day and 13.0 mg/kg bw per day for toddlers, respectively (Table 12).

The level of the impurities in the Primary Product combined with the estimated exposure to the Primary Product (Table 12) can be used to estimate the exposure to these impurities. This exposure can then be compared with reference points (RP, i.e. lower limit of the benchmark dose (BMDL) for arsenic, lead and PAH4) or health-based guidance values (HBGV, i.e. tolerable weekly intake (TWI) for cadmium and mercury) for the undesirable impurities present in the Primary Product (Table 13).

**Table 13:** Reference points/health-based guidance values for the impurities potentially present in the Primary Product

| Impurity/constituent/ HBGV/ RP | Basis/Reference |
|---|---|
| Arsenic (As)/ 0.3–8 μg/kg bw per day ($BMDL_{01}$) | The reference point is based on a range of benchmark dose lower confidence limit ($BMDL_{01}$) values between 0.3 and 8 μg/kg body weight (bw) per day identified for cancers of the lung, skin and bladder, as well as skin lesions MOE should be at least 10,000 if the reference point is based on carcinogenicity in animal studies. However, as the BMDL for As is derived from human studies, an interspecies extrapolation factor (i.e. 10) is not needed, i.e. a MOE of 1,000 would be sufficient (EFSA CONTAM Panel, 2009a; EFSA Scientific Committee, 2012). |
| Cadmium (Cd)/ 2.5 μg/kg bw per week (TWI) | The derivation of the reference point is based on a meta-analysis to evaluate the dose–response relationship between selected urinary cadmium and urinary beta-2-microglobulin as the biomarker of tubular damage recognised as the most useful biomarker in relation to tubular effects. A group-based $BMDL_5$ of 4 μg Cd/g creatinine for humans was derived. A chemical specific adjustment factor of 3.9 was applied to account for human variability in urinary cadmium within each dose-subgroup in the analysis resulting in a reference point of 1.0 μg Cd per g creatinine. In order to remain below 1 μg Cd/g creatinine in urine in 95% of the population by age 50. The average daily dietary cadmium intake should not exceed 0.36 μg Cd/kg bw. Corresponding to a weekly dietary intake of 2.5 μg Cd/kg bw (EFSA CONTAM Panel, 2009b). |
| Lead (Pb)/ 0.5 μg/kg bw per day ($BMDL_{01}$) | The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. The EFSA CONTAM Panel mentioned that a 1-point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1-point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOE lower than 1) (EFSA CONTAM Panel, 2010). |
| Mercury (Hg)/ 4 μg/kg bw per week (TWI) | The HBGV was set using kidney weight changes in male rats as the pivotal effect. Based on the $BMDL_{10}$ of 0.06 mg/kg bw per day, expressed as mercury, and an uncertainty factor of 100 to account for inter and intra species differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 μg/kg bw per week, expressed as mercury was established (EFSA CONTAM Panel, 2012). |
| PAH4/ 340 μg/kg bw per day ($BMDL_{10}$) | Polycyclic aromatic hydrocarbons (PAHs) are considered genotoxic and carcinogenic. The reference point is based on a carcinogenicity study by Culp et al. (1998), as reported by the EFSA CONTAM Panel (2008), who concluded that PAH4 (i.e. the sum of benzo[a]anthracene, benzo[a]pyrene, benzo[b] fluoranthene and chrysene) is a suitable indicator for the occurrence and toxicity of PAHs in food. The MOE should be at least 10,000 (EFSA CONTAM Panel, 2008). |

HBGV: health-based guidance value; RP: reference point; $BMDL_{01}$: lower confidence limit of the benchmark dose associated with a 1% extra risk for tumours (EFSA Scientific Committee, 2017a); $BMDL_{10}$: lower confidence limit of the benchmark dose associated with a 10% extra risk for tumours (EFSA Scientific Committee, 2017a); TWI: tolerable weekly intake; MOE: margin of exposure.

The risk assessment of the undesirable impurities helps to determine whether there could be a possible health concern if these impurities were present at their limit values in the Primary Product. The assessment is performed by calculating the MOE (margin of exposure) by dividing the reference point (i.e. BMDL, Table 13) by the exposure estimate for an impurity (Table 12), or by estimating the contribution of the exposure to an impurity due to the use of Primary Product to the HBGV (expressed as percentage of the HBGV).

#### 3.3.3.1. Toxic elements

The results of the analysis of arsenic, cadmium, lead and mercury in 19 batches of the Primary Product were reported (Table 3). The applicant proposed maximum limits for these toxic elements, as presented in Table 7. For cadmium the Panel noted that the proposed specification limits are lower than its highest measured value (see Table 3). The Panel assessed the risk that would result if these toxic elements were present in the Primary Product according to two concentration scenarios: (i) at

the current limits in the EU specifications and (ii) at the highest measured value for Cd multiplied by a factor of 5 by the Panel and the LOQs multiplied by a factor of 10 by the Panel for As, Hg and Pb; this to account for variability with respect to representativeness, homogeneity and analytical measurement.

The outcome of the risk assessment for the two concentration scenarios and based on the highest mean and the highest 95th percentile exposure estimates among the different population groups (see Section 3.3.2) is presented in Table 14.

**Table 14:** Risk assessment for four toxic elements present in the Primary Product according to two concentration scenarios, using the reference points/health-based guidance values as provided in Table 13

| | **(i) Considering the presence of toxic elements at the current EU specifications limits for Zesti Smoke Code 10** | | | |
|---|---|---|---|---|
| **Exposure to Zesti Smoke Code 10 (mg/kg bw per day)** | **MOE for As at3 mg/kg** | **% of the TWI for Cd at 1 mg/kg** | **MOE for Pb at5 mg/kg** | **% of the TWI for Hg at 1 mg/kg** |
| 4.6[(a)] | 21.7–580 | 1.3 | 21.7 | 0.8 |
| 13.0[(b)] | 7.7–205 | 3.6 | 7.7 | 2.3 |
| | **(ii) Considering the presence of toxic elements at the highest measured value for Cd multiplied by a factor of 5, and the LOQs multiplied by a factor of 10 by the Panel for As, Hg and Pb** | | | |
| | **MOE for As at 1 mg/kg** | **% of the TWI for Cd at 0.2 mg/kg** | **MOE for Pb at 0.5 mg/kg** | **% of the TWI for Hg at 0.05 mg/kg** |
| 4.6[(a)] | 65.2–1,739 | 0.3 | 217 | 0.04 |
| 13.0[(b)] | 23.1–615 | 0.7 | 76.9 | 0.1 |

bw: body weight; MOE: margin of exposure; TWI: tolerable weekly intake; LOQ: limit of quantification.
(a): Highest mean exposure level among the different population groups (proposed maximum use level exposure assessment scenario – toddlers (Table 12)).
(b): Highest 95th percentile exposure level among the different population groups (proposed maximum use level exposure assessment scenario – toddlers (Table 12)).

When considering the current limits of the EU specifications (scenario (i) in Table 14), the Panel concluded that for arsenic the ranges of the calculated MOE values were insufficient, i.e. below the target value of 1,000. For the other three toxic elements (cadmium, lead and mercury), the EU current specifications limit values do not give rise to safety concerns.

When considering the highest measured value for Cd multiplied by a factor of 5 and the LOQs multiplied by a factor of 10 for As, Hg and Pb (scenario (ii) in Table 14), the Panel concluded that for As (a) the lower end of the range for the highest mean and (b) the range for the highest 95th percentile of the calculated MOE values were insufficient, i.e. below the target value of 1,000. In this scenario, the presence of the other toxic elements in the Primary Product do not give rise to safety concern.

Overall, the Panel considered that the limits in the EU specifications for arsenic, cadmium, lead and mercury should be established based on actual levels in the commercial Primary Product. If the European Commission decides to revise the current limits in the EU specifications, the estimated exposure to the toxic elements as described above could be considered.

### 3.3.3.2. Polycyclic aromatic hydrocarbons (PAHs)

The results of the analysis of 16 PAHs were reported by the applicant for 19 batches of the Primary Product (Table 5).

The proposed limits for two of these PAHs (i.e. benzo[a]pyrene and benzo[a]anthracene) are below their respective limits of 10 and 20 μg/kg as laid down in Regulation (EC) No 2065/2003. However, the Panel noted that the actual measured levels for benzo[a]pyrene and benzo[a]anthracene in the Primary Product (Table 5) were substantially lower than the current limits in Regulation (EC) No 2065/2003.

According to the data submitted by the applicant, the Panel considered the maximum reported level of PAH4 (i.e. the sum of their LOQs) in the Primary Product, i.e. 2.0 μg/kg (Table 5). Based on this level, the Panel assessed the risk that would result if PAH4 were present in the Primary Product: (i) at

the regulatory limit for the sum of benzo[a]pyrene and benzo[a]anthracene in the Primary Product, i.e. 30 μg/kg, as laid down in Regulation (EC) No 2065/2003, and setting the concentration of the other two members of PAH4, (chrysene(a) and benzo[b]fluoranthene) at zero, for the purpose of this concentration scenario; and also (ii) at the maximum reported level of PAH4 in 19 batches of the Primary Product (Table 5).

The outcome of the risk assessment for the two concentration scenarios and based on the highest mean and the highest 95th percentile exposure estimates among the different population groups (see Section 3.3.2) is presented in Table 15.

**Table 15:** Risk assessment for PAH4, i.e. benzo[a]anthracene, benzo[a]pyrene, benzo[b] fluoranthene and chrysene in the Primary Product according to two concentration scenarios, using the reference points/health-based guidance values as provided in Table 13

| | **MOE for PAH4** |
|---|---|
| **Exposure to Zesti Smoke Code 10 (mg/kg bw per day)** | **(i) Considering the presence of PAH4 at the sum of the regulatory limits for benzo[a]pyrene and benzo[a] anthracene in Zesti Smoke Code 10 (30 μg/kg)** |
| 4.6[(a)] | $2.46 \times 10^6$ |
| 13.0[(b)] | $8.72 \times 10^5$ |
| | **(i) Considering the presence of PAH4 at their maximum reported level in Zesti Smoke Code 10 (2.0 μg/kg)** |
| 4.6[(a)] | $3.7 \times 10^7$ |
| 13.0[(b)] | $1.3 \times 10^7$ |

bw: body weight; MOE: margin of exposure.
(a): Highest mean exposure level among the different population groups (proposed maximum use level exposure assessment scenario – toddlers (Table 12)).
(b): Highest 95th percentile exposure level among the different population groups (proposed maximum use level exposure assessment scenario – toddlers (Table 12)).

The Panel concluded that the resulting MOEs for PAH4 were far above the target value of 10,000 for both concentration scenarios and both exposure estimates of the Primary Product (EFSA Scientific Committee, 2012) (Table 13).

Furthermore, the Panel noted that at the highest proposed maximum use level of the Primary Product in any of the food categories, i.e. 3,000 mg/kg food (Table 9), and the maximum reported level of PAH4 (i.e. the sum of their LOQs) in Primary Product, i.e. 2.0 μg/kg, the concentration of PAH4 in food would be $6.0 \times 10^{-3}$ μg/kg food, which is far below the lowest maximum level (ML) of these contaminants in any of the foods listed in Regulation (EU) 2023/915[11] (i.e. 1 μg PAH4/kg food).

## 3.4. Genotoxicity data

The present evaluation is conducted in line with the applicable EFSA guidance on smoke flavourings (EFSA FAF Panel, 2021) which encompasses all the EFSA guidance documents on genotoxicity (EFSA Scientific Committee, 2011, 2017b, 2019, 2021b). These documents were not available at the time when the smoke flavourings were evaluated previously by the CEF Panel. In addition, for the assessment of the renewal applications, the reliability and relevance of all submitted genotoxicity studies were evaluated by the FAF Panel (see Sections 3.4.1 and 3.4.2) based on the criteria, described in Appendix C.

### 3.4.1. Genotoxicity assessment of the individual components

The 50 identified components of Zesti Smoke Code 10 (SF-002) (i.e. 47 identified and quantified components as listed in the Appendix A, Table A.1 plus 3 identified, but not quantified, components as described in Section 3.1.2.4.2) were evaluated individually for potential concern of genotoxicity considering first the data available from the literature as provided by the applicant and then, in the absence of relevant information from the literature, considering the *in silico* information/data first submitted by the applicant and then generated by EFSA (see Annex B).

[11] Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. OJ L 119, 5.5.2023, p. 103–157.

Out of the 47 identified and quantified components, the applicant reported that 30 have already been evaluated by EFSA and/or JECFA/CoE and were concluded not to represent genotoxicity concern. For those components, the applicant relied on EFSA's conclusion on the genotoxic potential as set out in the respective Scientific Opinions of EFSA.

For one component, i.e. furan-2(5*H*)-one (CAS No.: 497-23-4; formerly [FL-no. 10.066]), EFSA previously concluded that, based on the available data, the substance is genotoxic *in vivo* (EFSA FAF Panel, 2019). The applicant further evaluated the risk with respect to genotoxicity for this substance using a risk-based approach (see further below in this section).

For three further components the applicant's conclusions were based on literature search, whereas the remaining 13 data-poor substances were assessed by means of read-across (grouping) considerations and/or *in silico* prediction of genotoxicity endpoints using a combination of independent and scientifically valid quantitative structure–activity relationship (QSAR ) models.

*In silico* data were generated by the applicant using the toxicity prediction tools Derek Nexus (version 6.2.0),[12] Leadscope Model Applier (version 3.0.2–4),[13] OECD QSAR Toolbox (version 4.5)[14] and VEGA (version 1.1.5-b47).[15]

The following models implemented in Derek Nexus were applied:

- Mutagenicity *in vitro* in bacterium
- Mutagenicity *in vitro* in *Escherichia coli*
- Mutagenicity *in vitro* in *Salmonella Typhimurium*
- Chromosome damage *in vitro*
- Chromosome damage *in vivo*
- Mutagenicity *in vivo*
- Non-specific genotoxicity *in vitro*
- Non-specific genotoxicity *in vivo*
- Photo-induced chromosome damage *in vitro*
- Photo-induced non-specific genotoxicity *in vitro*
- Photo-induced non-specific genotoxicity *in vivo*
- Photomutagenicity *in vitro*

The following models implemented in Leadscope Model Applier were applied:

- *In Vitro* Chrom Ab CHL v2
- *In Vitro* Chrom Ab CHO v2
- *In Vivo* Chrom Ab Comp v2
- *In Vivo* Chrom Ab Other v1
- *In Vivo* Chrom Ab Rat v1
- *In Vivo* Micronuc Mouse v2
- HGPRT Mut v1
- Bacterial Mutation v2

The Danish QSAR DB battery model (1.0) implemented in OECD QSAR Toolbox was applied. This includes the models as follows:

- Chromosome aberrations in Chinese Hamster Ovary (CHO) Cells
- Chromosome aberrations in Chinese Hamster Lung (CHL) Cells
- Micronucleus test in Mouse Erythrocytes
- Mutations in HGPRT Locus in Chinese Hamster Ovary (CHO) cells
- Comet Assay in Mouse

Eventually, the model *In Vitro* Micronucleus activity (IRFMN/VERMEER) 1.0.0 as implemented in VEGA was also used by the applicant to complement the *in silico* analysis.

More specifically, for three data-poor components, the applicant's conclusions were solely based on the results of the *in silico* models. For the remaining 10 data-poor substances read-across was performed by the applicant using congeneric surrogates identified by inspection of other smoke flavourings or by means of the OECD QSAR Toolbox. In cases of data gaps due to limited available

[12] https://www.lhasalimited.org/products/derek-nexus.htm
[13] https://www.instem.com/solutions/insilico/computational-toxicology.php
[14] https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
[15] https://www.vegahub.eu/

data for the surrogates for specific endpoints, *in silico* models were utilised for the target substances to provide additional information to conduct a weight of evidence evaluation. Read-across studies included a justification of similarity conducted by applying the following OECD QSAR Toolbox profilers:

- DNA alerts for AMES, Chromosomal Aberrations (CA) and Micronucleus (MN) by OASIS;
- DNA binding by OASIS;
- DNA binding by OECD;
- Protein binding alerts for chromosomal aberration by OASIS;
- *In vitro* mutagenicity (Ames test) alerts by ISS;
- *In vivo* mutagenicity (Micronucleus) alerts by ISS.

In addition, the similarity was further investigated by the applicant by means of key organic functional groups as derived from the 'Organic functional groups' empirical profiler of the OECD QSAR Toolbox and physicochemical properties derived or calculated from the OECD QSAR Toolbox.

For those components for which a concern for genotoxicity was identified by the applicant either from experimental genotoxicity data or from read-across/QSAR assessment as described above, a risk-based analysis was subsequently applied by the applicant based on a margin of exposure approach (Documentation provided to EFSA No. 1 and 3). According to the applicant, genotoxicity data can be amenable to dose–response modelling like other toxicological endpoints (Johnson et al., 2014) and no observed adverse effect levels (NOAELs) were derived from the available *in vivo* genotoxicity studies conducted either with the individual components or with the Primary Product (whole mixture). MOEs for genotoxicity were then calculated for each of these constituents, by comparing the derived NOAELs with the DietEx exposure estimates to each of the constituents via the consumption of the Primary Product (Documentation provided to EFSA No.1 and No.3).

The Panel noted that the derivation of NOAELs for DNA-reactive genotoxic substances is considered to be inappropriate; this also applies to mixtures containing such substances. Therefore, the Panel did not follow the NOAEL-approach suggested by the applicant. The Panel recognises ongoing developments to include dose–response modelling and derivation of benchmark dose levels in risk assessment strategies for such substances (Menz et al., 2023). However, the techniques as applied by the applicant (e.g. for furan-2(5*H*)-one, have not yet been validated and are not generally accepted.

A short summary of the data available from the literature as submitted by the applicant and of the overall conclusions from the applicant on the genotoxicity of the individual components, including the *in silico* analysis, when available, is reported in Annex B of this opinion (see columns 'G' and 'I'). The complete set of information from the applicant is available under the section 'Genotoxicity' of the technical dossier (see Documentation provided to EFSA No.1 and 3).

In line with the EFSA guidance on smoke flavourings (EFSA FAF Panel, 2021), the Panel conducted a (Q)SAR analysis for all the 50 identified components of the Primary Product using the six profilers as available in the OECD QSAR Toolbox v. 4.5, as listed above.

As described in column 'K' of Annex B, reporting the EFSA's conclusions on the genotoxicity of the components of the Primary Product based on the available data, the individual structural alerts identified by the six profilers may have different positive predictivity (i.e. rate of positives to the total number of substances with the alert) for the genotoxicity of the target substance. The concepts of the alerts are described by the European Chemicals Agency (ECHA, 2008) and the predictivities of the individual alerts are documented by Benigni et al. (2008, 2009). When necessary, the application of profilers was followed by an expert review (e.g. check of close analogues/structurally related substances).

Overall, regarding the genotoxicity assessment of the individual components of the Primary Product the Panel noted that:

i) for 42 identified components, based on the (often limited) genotoxicity data available from the literature either on the substance or on structurally related substances, the Panel concluded that the data did not indicate a concern for genotoxicity (see Annex B).
ii) for one substance, levoglucosane (CAS No. 498-07-7), no genotoxicity data were available. Regarding the (Q)SAR analysis, a weak indication for potential genotoxicity was identified for one of the profilers, i.e. '*in vivo* mutagenicity (Micronucleus) alerts by ISS: H-acceptor-path3-H-acceptor' (see Annex B). However, no structural alerts for genotoxicity were identified by any of the other five profilers of the OECD QSAR Toolbox. Together with the consideration that the ring size (6-membered) of the oxane, resulting from the dehydration

of glucose, indicates stability of the molecule, the indication for genotoxicity of this target substance based on (Q)SAR analysis is alleviated and it is not further considered.

iii) for one substance, (*S*)-2,3-dihydroxypropanal (CAS No. 497-09-6), positive *in vitro* experimental data (mutagenicity) are available, as reported by the applicant (Yamaguchi, 1982; Yamaguchi and Nakagawa, 1983). Nevertheless, the target substance (also known as glyceraldehyde) is an intermediate in the metabolism of glucose and fructose. Consequently, endogenous amounts are orders of magnitude larger than the exposure resulting from its presence in smoke flavourings. Therefore, the Panel concluded that the exposure to the target substance resulting from the consumption of food containing this smoke flavouring does not raise a safety concern.

iv) for two components, i.e. furan-2(5*H*)-one (CAS No. 497-23-4, former [FL-no: 10.066]) and benzene-1,2-diol (CAS No. 120-80-9), [FL-no: 04.029]), the Panel identified a concern for genotoxicity (see Annex B and Appendix B).

v) for 4 components, i.e. hydroxyacetaldehyde (CAS No. 141-46-8), acetaldehyde (CAS No. 75-07-0), formaldehyde (CAS No. 50-00-0) and 2-propenal (acrolein, CAS No. 107-02-8), the Panel identified potential concern for genotoxicity for which additional data would be needed to reach a final conclusion on the genotoxic potential of these substances (see Annex B and Appendix B).

The Panel investigated if the concern for genotoxicity for furan-2(5*H*)-one and benzene-1,2-diol and the potential concern for genotoxicity for the four components listed in (iv) could be ruled out by application of the threshold of toxicological concern (TTC) approach for DNA-reactive mutagens and/or carcinogens (EFSA Scientific Committee, 2019). For this purpose, the Panel calculated the exposure to each of these components by multiplying the estimated exposure to the Primary Product (proposed maximum use level exposure assessment scenario, estimated with DietEx – Table 12) by the average content of these components in the Primary Product (see Appendix A). For 2-propenal (acrolein), this could not be done because this substance was not quantified in the Primary Product by the applicant.

The obtained exposure estimates for the remaining five substances were compared with the TTC value of 0.0025 μg/kg bw per day for DNA-reactive mutagens and/or carcinogens. All exposure estimates were at least a factor of 10,400 above this TTC value (see Table 16) and therefore the application of the TTC approach could not rule out the (potential) concern for genotoxicity for these components.

The lack of robust experimental data on genotoxicity for the four components listed in (v) for which a potential concern for genotoxicity was identified is a non-standard uncertainty with respect to the genotoxicity assessment of the individual components (see Section 2.2 of this opinion and Table G.1 of the EFSA guidance document on smoke flavouring (EFSA FAF Panel, 2021)). This uncertainty can only be addressed with additional genotoxicity data.

**Table 16:** Dietary exposure in μg/kg body weight (bw) per day to five individual components[(a)] for which a (potential) concern for genotoxicity has been identified (see Appendix B), based on the proposed maximum use level exposure assessment scenario using DietEx (Table 12)

| CAS No. | Chemical Name | Average content in the Primary Product (wt%) | Exposure | Infants (12 weeks–11 months) | Toddlers (12–35 months) | Children (3–9 years) | Adolescents (10–17 years) | Adults(18–64 years) | The elderly (≥ 65 years) | Ratio between the highest exposure estimate and TTC |
|---|---|---|---|---|---|---|---|---|---|---|
| **Components of concern for genotoxicity** | | | | | | | | | | |
| 120-80-9 | benzene-1,2-diol (1,2-benzenediol, catechol) | 0.3 | Mean | 0.0–3.6 | 1.2–13.8 | 1.5–12.6 | 1.2–6.3 | 1.2–4.8 | 0.6–3.9 | $1.56 \times 10^4$ |
| | | | 95th percentile | None–18.3 | 5.7–39.0 | 8.1–36.9 | 7.5–18.0 | 4.8–15.9 | 2.4–12.3 | |
| 497-23-4 | furan-2(5*H*)-one (2(5*H*)furanone) | 0.3 | Mean | 0.0–3.6 | 1.2–13.8 | 1.5–12.6 | 1.2–6.3 | 1.2–4.8 | 0.6–3.9 | $1.56 \times 10^4$ |
| | | | 95th percentile | None–18.3 | 5.7–39.0 | 8.1–36.9 | 7.5–18.0 | 4.8–15.9 | 2.4–12.3 | |
| **Components for which a potential concern for genotoxicity is identified** | | | | | | | | | | |
| 141-46-8 | hydroxyacetaldehyde | 2.4 | Mean | 0.0–28.8 | 9.6–110.4 | 12.0–100.8 | 9.6–50.4 | 9.6–38.4 | 4.8–31.2 | $1.248 \times 10^5$ |
| | | | 95th percentile | None–146.4 | 45.6–312.0 | 64.8–295.2 | 60.0–144.0 | 38.4–127.2 | 19.2–98.4 | |
| 75-07-0 | acetaldehyde | 0.4 | Mean | 0.0–4.8 | 1.6–18.4 | 2.0–16.8 | 1.6–8.4 | 1.6–6.4 | 0.8–5.2 | $2.08 \times 10^4$ |
| | | | 95th percentile | None–24.4 | 7.6–52.0 | 10.8–49.2 | 10.0–24.0 | 6.4–21.2 | 3.2–16.4 | |
| 50-00-0 | formaldehyde | 0.2 | Mean | 0.0–2.4 | 0.8–9.2 | 1.0–8.4 | 0.8–4.2 | 0.8–3.2 | 0.4–2.6 | $1.04 \times 10^4$ |
| | | | 95th percentile | None–12.2 | 3.8–26.0 | 5.4–24.6 | 5.0–12.0 | 3.2–10.6 | 1.6–8.2 | |

wt: weight; TTC: threshold of toxicological concern.
(a): A sixth substance, 2-propenal, was not included in this table since its concentration in the Primary Product was not determined.

### 3.4.2. Genotoxicity assessment of the Primary Product (whole mixture)

The applicant resubmitted the genotoxicity studies on the Primary Product (whole mixture) that were already evaluated by the CEF Panel in 2009, to investigate the genotoxicity of the unidentified fraction of the Primary Product, in line with the EFSA Scientific Committee statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019): a bacterial reverse mutation test (Covance, 2004), an *in vitro* mammalian cell gene mutation assay in mouse lymphoma cells (Covance, 2006a), an *in vitro* mammalian chromosomal aberration test (Covance, 2006b), an *in vivo* micronucleus assay in mouse bone marrow (Covance, 2006c) and an *in vivo* rat liver unscheduled DNA synthesis (UDS) assay (Covance, 2005).

The evaluation of these studies as described in the scientific opinion 'Safety of smoke flavour Primary Product – Zesti Smoke Code 10' (EFSA CEF Panel, 2009) is reported in Section 3.4.2.1. For each study, comments and evaluation by the FAF Panel are reported. These studies are summarised in Tables D.1 and D.2 (Appendix D), where the evaluation of reliability and relevance are reported (according to the approach described in Appendix C).

The Panel noted that the general compositional data of the product evaluated in 2009 do not fundamentally deviate from the product assessed in the current opinion. In addition, as stated by the applicant, the manufacturing process has not changed and the batch-to-batch variability was low both in the previous evaluation (EFSA CEF Panel, 2009) and in the current opinion (see Table 6 in Section 3.1.2.6). Therefore, the Panel considered the Primary Product that was evaluated in 2009 similar to the Primary Product evaluated in this opinion and that the batch used for the genotoxicity testing in the past can still be considered representative for the current product.

In addition, new *in vitro* and *in vivo* genotoxicity studies were provided, which are described in Section 3.4.2.2 and summarised in Appendix E.

The batch used in these newly submitted genotoxicity studies (no. 05210465) fell within the reported range of batch-to-batch variability and is considered representative (see Section 3.1.3).

The Panel noted that information provided to confirm the absence of a fraction of small particles is not sufficient (see Section 3.1.2.7). Therefore, the conclusions reached for each of the genotoxicity studies described below is applicable only under the assumption that the material is covered by the conventional risk assessment and does not require a separate assessment regarding nanoscale properties.

#### 3.4.2.1. Studies evaluated in EFSA CEF Panel opinion (EFSA CEF Panel, 2009)

*3.4.2.1.1. Bacterial reverse mutation test (Covance, 2004)*

*'Primary Product Zesti Smoke Code 10 was assayed for mutagenicity in 5 histidine-requiring strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537 and TA102) in the absence and presence of metabolic activation up to the recommended limit concentration of 5,000 μg/plate (..). Primary Product Zesti Smoke Code 10 showed no cytotoxicity or precipitation within the test system. A statistically significant ($p < 0.005$) increase in the number of revertant colonies was seen at the top concentration tested (5,000 μg/plate) in the range-finder experiment using TA100 (1.37-fold increase without S9 and 1.62-fold increase with S9-fold). Statistically significant (Dunnett's test to compare the counts of each dose with the control and linear regression analysis to test for the presence of a dose response), dose-related and reproducible increases in revertant numbers were observed following treatment of strain TA100 at concentrations of 1,000 μg/plate and above in the absence and presence of S9 up to 1.62-fold ($p < 0.005$) and 1.40-fold ($p < 0.005$) increase in the absence and presence of S9 at 5,000 μg/plate. Statistically significant, dose-related and reproducible increases in revertant numbers were also observed following treatment of the closely related strain TA1535 at concentrations of 1,000 μg/plate (1.21-fold increase) ($p < 0.05$) and at 5,000 μg/plate (2.16-fold increase) ($p < 0.005$) in the absence of S9 and at the top concentration tested (5,000 μg/plate) in the presence of S9 (1.51-fold increase) ($p < 0.005$). In TA102 a dose-dependent statistically significant increase up to a 1.45-fold increase ($p < 0.005$) without S9 and a 1.39-fold increase ($p < 0.01$) in the presence of S9 at 5,000 μg/plate was observed (Batch no BJW 3842). The applicant concludes that Primary Product Zesti Smoke Code 10 showed weak mutagenic activity in this test system'.* (EFSA CEF Panel, 2009).

The FAF Panel agreed with the conclusions on the weak mutagenic activity, considering the concentration-response trend and the reproducible increase in the number of revertants reported for the strains TA100, TA102 and TA1535. Accordingly, the FAF Panel considered this study as positive, reliable without restrictions and the results of high relevance.

*3.4.2.1.2. In vitro* mammalian cell gene mutation assay in mouse lymphoma cells (Covance, 2006a)

*'Primary Product Zesti Smoke Code 10 was assayed for its ability to induce mutation at the tk locus in mouse lymphoma cells (..). The highest concentrations analysed were selected on the basis of cytotoxicity and amounted to 225 μg/mL in the absence of S9 and 450 μg/mL in the presence of S9 which yielded 24% and 16% relative survival, respectively. Statistically significant increases in mutant frequency were observed following treatment with Primary Product Zesti Smoke Code 10 at the top three concentrations analysed (150–225 μg/mL) in the absence of S9 and at the top four concentrations analysed (300–450 μg/mL) in the presence of S9. Highly significant linear trends were also obtained in both the absence and presence of S9. At concentrations where a statistically significant increase in mutant frequency was observed, increases in both small and large colony mutant frequencies were observed. The applicant concludes that Primary Product Zesti Smoke Code 10 was genotoxic in this test system'*. (EFSA CEF Panel, 2009).

The FAF Panel agreed with the previous evaluation of the CEF Panel (EFSA CEF Panel, 2009) that the Primary Product gave clear positive results in all test conditions. The study is positive also when applying the global evaluation factor as an additional criterion to evaluate the results as recommended in the current OECD TG 490 (OECD, 2016a). The study was considered reliable without restriction and the results are of high relevance.

*3.4.2.1.3. In vitro* mammalian chromosomal aberration test (Covance, 2006b)

*'Primary Product Zesti Smoke Code 10 (BJW 3842) was tested in an in vitro cytogenetic assay with cultured human peripheral blood lymphocytes in the absence and presence of metabolic activation (S9) up to the acceptable maximum concentration of 5,000 μg/mL (..). Primary Product Zesti Smoke Code 10 was tested using a 3-hour treatment period with a 17-h recovery period (3 + 17 h) in both the absence and presence of S9 and using an extended treatment (20 + 0 h) in the absence of S9. The concentrations chosen for analysis for chromosome aberrations ranged from 219.9 to 429.5 μg/mL (-S9, 3 + 17 h), 219.9 to 737.0 μg/mL (+S9, 3 + 17 h) and 313.2 to 433.5 μg/mL (-S9, 20 + 0 h) with the highest concentrations showing appropriate levels of toxicity (approximately 40% to 62% mitotic inhibition [reduction in mitotic index]). For some of the concentrations analysed less than the intended 200 cells (made up of 100 cells per replicate) were analysed*.

*However, at least 184 cells out of an intended 200 were analysed at each dose level. Treatment of cultures with Primary Product Zesti Smoke Code 10 in the absence and the presence of S9 resulted in frequencies of cells with structural aberrations, which were generally elevated compared to those observed in concurrent negative control cultures. For the majority of treatment conditions, the increases in the frequencies of cells with aberrations were statistically significant with numbers of cells with aberrations up to 41/200 (41 out of 200 cells) at 429.5 μg/mL (–S9, 20 + 0 h)*[16] *versus 1/200 for the corresponding control ($p < 0.0001$), 62/188 at 737.0 μg/mL (+S9, 3 + 17 h) versus 2/200 for the corresponding control ($p < 0.001$) and 65/190 at 433.5 μg/mL (-S9, 20 + 0 h) versus 1/200 for the corresponding control ($p < 0.001$)*[17]*. The applicant concludes that Primary Product Zesti Smoke Code 10 had the potential to induce chromosome aberrations in cultured human peripheral blood lymphocytes in both the absence and presence of S9'*. (EFSA CEF Panel, 2009).

The FAF Panel agreed with the CEF Panel that the Primary Product showed evidence of clastogenic activity both in the absence and in the presence of metabolic activation. However, based on the most recent OECD TG 473 (OECD, 2016b) the study is considered as reliable with restrictions, because only 200 or less metaphases/concentration instead of 300 were scored. Furthermore, no historical ranges for positive controls were reported. Therefore, the Panel considered that the study results are of limited relevance.

*3.4.2.1.4. In vivo* bone marrow mouse micronucleus test (Covance, 2006c)

*'Primary Product Zesti Smoke Code 10 (Batch no BJW 0345) was also assayed in vivo in a rat bone marrow micronucleus test at a dose level of 2,000 mg/kg bw (the acceptable limit dose for a nontoxic material) (..). The test article was administered via oral gavage once daily on two consecutive days to a group of six male Sprague Dawley Crl:CD®(SD) rats at a single dose of 2,000 mg/kg bw per day. The animals were sacrificed 24 h after the second administration. Male rats treated with Primary Product Zesti Smoke Code 10 exhibited a group mean % polychromatic erythrocytes (PCE ) [42%]*

[16] The correct test condition is 3-17 h without S9-mix.
[17] The data reported are related to chromosomal aberrations including gaps, however, the outcome was positive also when gaps were excluded.

*that was lower than the value for the vehicle control group [50%] and which fell below the lower limit of the historical 95% confidence interval (48–67%). The applicant indicates that this may be an indication of bone marrow toxicity and exposure, although there was some variability in %PCE between individual animals. The frequencies of micronucleated PCE (MN PCE) in the test article treated group were not significantly different from those observed in the concurrent vehicle control group and individual animal MN PCE frequencies were consistent with historical distribution data*.

*The applicant concludes that Primary Product Zesti Smoke Code 10 did not induce micronuclei in the PCE of the bone marrow of male rats treated at 2,000 mg/kg bw per day. The Panel notes that the bone marrow micronucleus test was a valid study as it was conducted using the acceptable limit dose of 2,000 mg/kg bw. However, in order to be confident about a negative result, evidence of adequate exposure of the target cells is required. No plasma analysis was conducted during the in vivo studies (due to practical constraints – Primary Product Zesti Smoke Code 10 is a complex mixture). However, a reduction in the PCE to NCE (normochromatic erythrocytes) ratio (42% compared to 50% for vehicle control) was observed and this may be an indication of bone marrow toxicity and hence exposure (although some variability in % PCE was noted between animals)'*. (EFSA CEF Panel, 2009).

The FAF Panel agreed that the Primary Product did not induce micronuclei in this rat bone marrow MN test. However, higher doses[18] could have been applied.

Furthermore, the Panel noted that the assay was conducted at one dose level of 2,000 mg/kg bw.

The study design in which only one dose was used was in line with the OECD TG 474 (OECD, 1997) because the limit dose of 2,000 mg/kg bw was applied. The dosing at limit dose only is also possible according to the OECD TG 474 version from 2016 (OECD, 2016c), however, only if there is no indication of genotoxicity from *in vitro* tests, which is not the case here as there was *in vitro* genotoxicity.

In addition, the limit dose of 2,000 mg/kg bw is applicable for the testing of chemicals, but for mixtures such as smoke flavourings Primary Products additional considerations are needed.

The Panel is of the view that if no toxicity is observed in an appropriately designed range-finding study with a Primary Product, it would be appropriate to test higher doses than 2,000 mg/kg bw, 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 (OECD, 2016c) and 489 (OECD, 2016d), 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.[18]

The Panel noted further that the study was performed according to OECD TG 474 (OECD, 1997) but that, according to the more recent version of this TG from 2016, 4,000 instead of 2,000 cells should have been scored for micronuclei. The Panel also noted that no clinical signs of toxicity or marked loss of body weight was observed in any animal and that the reduction in the ratio of PCE to NCE was less than 20% of that measured for the negative control group which is only moderate. It should also be noted that, according to the statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019), even if there were demonstration of bone marrow exposure, the assessment of genotoxicity of mixtures in the bone marrow is limited by the fact that target tissue exposure to all potential genotoxic components cannot be demonstrated unequivocally.

The study was negative in the experimental conditions applied, but since no evidence of target tissue exposure was provided, this assay is evaluated as inconclusive. The Panel considered the study as reliable with restrictions and the study result of low relevance.

*3.4.2.1.5. In vivo* rat liver UDS assay (Covance, 2005)

*'Primary Product Zesti Smoke Code 10 (Batch BJW 0345) was tested for its ability to induce unscheduled DNA synthesis (UDS) in the livers of orally dosed male rats using an in vivo/in vitro procedure (..). The UDS assay is generally considered to show limited sensitivity to compounds which induce either point mutations or large deletions due to the nature of the endpoint (radiolabelling of sections of repaired DNA) used for detection of genotoxicity. However, there was evidence from both the mouse lymphoma assay (MLA) and the chromosome aberration test in vitro that Primary Product Zesti Smoke Code 10 induced clastogenic DNA damage in these studies, which would be detected by the UDS assay. Groups of four male Sprague Dawley Crl:CD®(SD) rats were treated once with the*

[18] https://www.efsa.europa.eu/sites/default/files/2021-10/24th-plenary-meeting-faf-panel-proposed-be-open-observers-minutes.pdf

*vehicle (15% Tween 80 in purified water), Primary Product Zesti Smoke Code 10 (at the acceptable limit dose of 2,000 mg/kg bw) or the required positive control, via oral gavage, approximately 12–14 h (Experiment 1) or 2–4 h (Experiment 2) after dosing, animals were sacrificed, and their livers perfused with collagenase to provide a primary culture of hepatocytes. Cultures were made from three animals in each dose group and were treated with [$^3$H] thymidine. There were no clinical signs or effects on body weight observed in any animal treated with Primary Product Zesti Smoke Code 10 in the main study. The applicant indicates that because Primary Product Zesti Smoke Code 10 is a complex mixture, confirmation of systemic exposure (via analysis of plasma) was not considered to be relevant, and that these assessments were not performed in this UDS study. Treatment with 2,000 mg/kg flavouring Primary Product Zesti Smoke Code10 did not produce a group mean net nuclear grain (NNG) value greater than 0.5 nor were any more than 4.3% cells found in repair at either harvest time point (2–4 h and 12–14 h post dose). It was concluded that Primary Product Zesti Smoke Code 10 did not induce UDS detectable under the experimental conditions employed in this study'*. (EFSA CEF Panel, 2009).

The FAF Panel agreed with the previous evaluation of the CEF Panel. However, based on the low adequacy of the UDS assay to follow-up positive *in vitro* results, as explained in the EFSA Scientific Committee Opinion (EFSA Scientific Committee, 2017b), the Panel considered that the results of a negative UDS study are of low relevance and, accordingly, do not contribute to the overall assessment of genotoxicity.

#### 3.4.2.2. New genotoxicity studies

Based on the available data and on the requirements of the EFSA guidance on smoke flavouring Primary Products (EFSA FAF Panel, 2021) new genotoxicity studies were submitted: a bacterial reverse mutation test (Labcorp, 2021a), an *in vitro* MN test (Labcorp, 2021b), an *in vivo* MN test in bone marrow (Labcorp, 2022a) and an *in vivo* gene mutation study in transgenic mice (Labcorp, 2022b).

By measuring the concentration of 2,6-dimethoxyphenol, a typical component of the Primary Product, (using LC MS/MS as described in Labcorp, 2021c), in liquid vehicles and diet, the applicant confirmed the concentrations of the Primary Product used in the *in vitro* and *in vivo* genotoxicity studies.

##### *3.4.2.2.1. Bacterial reverse mutation assay*

A bacterial reverse mutation assay was conducted in *S*. Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 to assess the mutagenicity of Zesti Smoke Code 10 (batch no. 05210465), both in the absence and in the presence of metabolic activation by β-naphthoflavone/phenobarbital-induced rat liver S9 fraction (S9-mix). Two separate experiments were conducted, Experiment 1 using the plate incorporation method and Experiment 2 using the preincubation method (Labcorp, 2021a). Study design complied with OECD TG 471 (OECD, 2020a) and with good laboratory practices (GLP) principles.

Positive control chemicals and dimethyl sulfoxide (DMSO) as vehicle control were evaluated concurrently. Both experiments were conducted in triplicate plates.

In Experiment 1, Zesti Smoke Code 10 was tested at seven concentrations ranging from 5 to 5,000 μg/plate, and in Experiment 2 at eight concentrations ranging from 50 to 5,000 μg/plate for all strains. No precipitation was observed in either experiment.

In both experiments, positive control chemicals induced significant increases in revertant colony numbers and mean revertant colony numbers for the vehicle controls were within the respective historical control ranges. A few individual vehicle control counts fell outside the laboratory's control ranges but only slightly and were considered by the study author not to invalidate the study results.

A reduction in background bacterial lawn growth for plates incubated with the test item was not observed in Experiment 1, but was observed in Experiment 2 in the absence of S9-mix at 2,000 μg/plate and above for strain TA98, at 5,000 μg/plate for strain TA100, at 3,500 μg/plate and above for strain TA1535 and both in the absence and presence of S9-mix at 5,000 μg/plate for strain TA1537.

In Experiment 1 (plate incorporation assay), increases in revertant colony numbers were observed at 5,000 μg/plate in strain TA102 in the presence of S9-mix compared to the concurrent vehicle control, which exceeded the 1.5-fold increase threshold. Furthermore, strain TA102 in the absence of S9-mix and TA100 in the presence of S9-mix showed concentration-related increases (not reaching the respective thresholds) in the mean number of revertant colonies.

In Experiment 2 (preincubation assay), ≥ 1.5-fold increases were observed for strain TA102 at 3,500 μg/plate, but not at 5,000 μg/plate in the absence of S9-mix and at 2,000 μg/plate and above

in the presence of S9-mix. Also, a two-fold increase in revertant numbers was almost reached for strain TA100 at 5,000 μg/plate in the presence of S9-mix (271.7) compared to concurrent vehicle control (136.7).

An apparent concentration-response effect was observed in both the experiments and the upper level of the corresponding historical control range was exceeded.

The study authors concluded that under the experimental conditions reported, Zesti Smoke Code 10 was mutagenic.

The Panel agreed with this conclusion and considered the study as reliable without restrictions and the positive results of high relevance.

*3.4.2.2.2. In vitro* mammalian cell micronucleus test

An *in vitro* MN test, with cytokinesis block protocol, was carried out according to OECD TG 487 (OECD, 2016e) and in compliance with GLP. Human peripheral blood lymphocytes from healthy donors were treated with Zesti Smoke Code 10 (batch no. 05210465) (Labcorp, 2021b).

Treatments started after a 48 h stimulation period with phytohaemagglutinin. A single experiment tested the following exposure conditions: 3 h exposures with and without metabolic activation by β-naphthoflavone/phenobarbital-induced rat liver S9 fraction (S9-mix), followed by a 21 h recovery period in the presence of cytochalasin B and a 24 h exposure without S9-mix and without cytochalasin B followed by a 24 h recovery period in the presence of cytochalasin B. The Panel noted that the extended treatment exposure conditions differed from the suggested cell treatment schedule in OECD TG 487 (OECD, 2016e). However, the Panel considered that the protocol applied for the extended treatment could potentially enhance the sensitivity of the MN test (Whitwell et al., 2019). Therefore, the Panel did not consider this aspect as a limitation.

Cyclophosphamide, mitomycin C and vinblastine were used as the positive controls and DMSO was used as the vehicle control. For each experimental condition, two cultures were analysed in parallel (Labcorp, 2021b).

A cytotoxicity range finding experiment was carried out with a range of concentrations up to 5,000 μg/mL for all exposure conditions.

For the MN experiment, lymphocytes were treated with Zesti Smoke Code 10 with 12–13 concentrations ranging from 60 to 500 μg/mL in the 3 h treatments both in the absence and in the presence of S9-mix and with 12 concentrations from 60 to 300 μg/mL in the 24 h treatment in the absence of metabolic activation. No precipitate of the test item was noted in any of the exposure conditions.

The replication index cytotoxicity data were used to select the concentrations for the MN analysis.

In the treatment of 3 h + 21 h in the absence of S9-mix, the following concentrations were chosen for the MN analysis: 120, 225 and 325 μg/mL (cytotoxicity of 10%, 28% and 50%, respectively). The mean frequency of bi-nucleated cells with micronuclei (MNBN) at the top concentration (2.65%) was statistically significantly increased compared to the vehicle control (0.55%) and increases in MNBN frequency were concentration-related (trend test: $p < 0.001$). Furthermore, MNBN frequencies at both the intermediate and the top concentrations exceeded the 95% reference range of the historical vehicle control (0.10–0.70%).

In the treatment of 3 h + 21 h in the presence of S9-mix, the following concentrations were chosen for the MN analysis: 140, 270, 350 and 400 μg/mL (cytotoxicity of 9%, 27%, 47% and 60%, respectively). The mean frequency of MNBN at all concentrations (1.15%, 1.00%, 2.15% and 2.85%, respectively) was statistically significantly increased compared to the vehicle control (0.45%) and increases in MNBN frequency were concentration-related (trend test: $p < 0.001$). Furthermore, mean MNBN frequencies at all concentrations exceeded the 95% reference range of the historical vehicle control (0.10–0.80%).

In the treatment of 24 h + 24 h in the absence of S9-mix, the following concentrations were chosen for the MN analysis: 120, 180, 200 and 220 μg/mL (cytotoxicity of 10%, 36%, 46% and 64%, respectively). The mean frequency of MNBN cells at all concentrations (4.25%, 9.05%, 10.55% and 13.50%, respectively) was statistically significantly increased compared to the vehicle control (0.65%) and increases in MNBN frequency were concentration-related(trend test: $p < 0.001$). Furthermore, mean MNBN frequencies at all concentrations exceeded the 95% reference range of the historical vehicle control (0.10–0.70%).

In all three test conditions, Zesti Smoke Code 10 induced statistically significant increases in the mean frequency of micronucleated cells compared to vehicle controls and also concentration-

dependent trends. Therefore, the authors of this study concluded that Zesti Smoke Code 10 induced micronuclei in human peripheral blood lymphocytes, under the conditions of this study.

The Panel agreed with this conclusion and considered the study reliable without restrictions and the positive result of high relevance.

Results of *in vitro* studies are summarised in Appendix E, Table E.1.

*3.4.2.2.3. In vivo* mammalian erythrocyte micronucleus test

Zesti Smoke Code 10 (batch no. 05210465) was tested in a bone marrow MN assay in rats, which was performed in compliance with GLP and according to OECD TG 474 (OECD, 2016c) (Labcorp, 2022a).

A dose range finding experiment was performed to identify the appropriate maximum dose level for the main test. Groups of three male and three female Han Wistar rats were treated twice at 24 h intervals by oral gavage at 3,500 and 5,000 mg/kg bw per day. In the top dose group, two females and one male died, and in all the others, piloerection and reduced activity were noted. In the 3,500 mg/kg bw per day group there were no deaths, but animals had a slight reduction in body weight and clinical signs that had ceased by day 3. Therefore, 3,500 mg/kg bw per day was considered the maximum tolerated dose (MTD). The MTD was used as the highest dose level in the main study. As no sex differences were observed in this experiment, the main experiment was performed in male rats only. At all doses, PCE/NCE ratios showed no evidence of bone marrow toxicity.

In the main study, groups of six male Han Wistar rats were treated via oral gavage with Zesti Smoke Code 10 at doses of 0 (vehicle control: deionised water), 875, 1,750 or 3,500 mg/kg bw per day. Test item formulations were used within 2 days of preparation. Animals were dosed at 0 and 24 h. A single administration (at 24 h) of 20 mg/kg bw cyclophosphamide via oral gavage was used as positive control (three rats only).

For four animals from the top dose group, a mild decrease in activity was observed after the second dose that was absent by day 3. No clinical signs were observed in the other groups.

Approximately 24 h after the final administration, animals were euthanised and femoral bone marrow was harvested and prepared for the MN analysis. A total of at least 500 PCE and NCE were scored to assess potential bone marrow toxicity. For the MN analysis, 4,000 PCE per animal were scored for the presence of MN.

The mean vehicle control data on micronucleated polychromatic erythrocytes (MNPCE) were comparable with the laboratory's historical vehicle control (within 95% reference range) data. Mean positive control data resulted in a statistically significant increase in MNPCE compared to the concurrent vehicle control, which was comparable with the laboratory's historical positive control (within 95% reference range) data.

In all three dose groups of rats treated with Zesti Smoke Code 10, there were no statistically significant increases in MNPCE mean frequency compared to the vehicle control and frequencies were also within the 95% reference range for the historical vehicle control (0.03–0.32%). Individual frequencies of MNPCE for all treated animals were also consistent with historical vehicle control data, with the exception of one 1,750 mg/kg bw per day group animal that had a MNPCE frequency of 0%.

The PCE/NCE ratios were not affected by treatment with Zesti Smoke Code 10 and they also fell within the laboratory's 95% reference range for the historical vehicle control.

To demonstrate bone marrow exposure of rats treated with Zesti Smoke Code 10, a plasma analysis for a satellite group was performed. Analysis of the plasma concentration of the constituent 2,6-dimethoxyphenol was used to monitor systemic exposure. According to the study authors, results of the bioanalysis demonstrated the presence of the 2,6-dimethoxyphenol in plasma and confirmed that animals were systemically exposed to Zesti Smoke Code 10.

The study authors concluded that Zesti Smoke Code 10 did not induce micronucleated erythrocytes in rat bone marrow cells under the conditions of this study (Labcorp, 2022a).

The Panel agreed with this conclusion, but considered that the plasma analysis of a marker constituent to demonstrate bone marrow exposure to individual constituents of a complex mixture provides insufficient information, since toxicokinetic characteristics of different constituents can be anticipated to show large differences. In addition, for this Primary Product a major part of the composition is not identified, which further hampers the applicability of marker substances to monitor target tissue exposure.[18]

Furthermore, the Panel noted that the PCE/NCE ratios were not affected by treatment with Zesti Smoke Code 10 and that the clinical signs observed in animals of the top dose group cannot be considered as line of evidence of systemic exposure.

It should also be noted that, according to the statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019), even if there were demonstration of bone marrow exposure, the assessment of genotoxicity of mixtures in the bone marrow is limited by the fact that target tissue exposure to all potential genotoxic components cannot be demonstrated unequivocally.

Accordingly, the Panel considered the study reliable with restrictions and the inconclusive result of low relevance.

*3.4.2.2.4. In vivo* gene mutation assay in Muta™Mouse transgenic mice

Zesti Smoke Code 10 (batch no. 05210465) was tested in a 15-day dose range finding (DRF) (non-GLP) study in Muta™Mouse ($CD_2$-lacZ80/HazfBR), once daily via oral gavage dosing or via the diet, in order to determine the MTD and dose levels for the transgenic rodent (TGR) gene mutation assay using the same rodent strain (Labcorp, 2022c). Bioanalyses were also performed on additional groups in order to confirm the appropriate dose route.

Zesti Smoke Code 10 was administered via oral gavage (vehicle control: deionised water) or via the diet (vehicle control: 5 KB3 Rodent Diet) *ad libitum* to groups of Muta™Mouse animals (three animals per sex per group). Dose levels were 700, 1,000 (males only), 1,400 and 2,000 (top dose females only) mg/kg bw per day for oral gavage administration and feed concentrations were 25,000, 35,000 and 50,000 mg/kg diet. The concentrations in the diet corresponded to mean achieved dose levels of 4,386, 6,441 and 7,684 mg/kg bw per day in males and 5,160, 8,388 and 9,952 mg/kg bw per day in females.

For the bioanalyses experiment, performed as part of the DRF study, (six animals per group, except for five animals in the female oral gavage group), 700 (males only) and 1,400 mg/kg bw per day (females only) and 35,000 (males only) and 50,000 mg/kg diet (females only) doses were given for seven consecutive days. The concentrations in the diet corresponded to mean achieved dose levels of 5,430 mg/kg bw per day for males and 10,491 mg/kg bw per day for females. Analysis of the plasma concentration of the constituent 2,6-dimethoxyphenol was used to monitor systemic exposure. For the oral gavage dosed groups, concentrations of 2,6-dimethoxyphenol, the marker analyte, were present in the highest concentrations 5 min post dose in both males and females (178 and 699 ng/mL, respectively). For the diet dosed groups, the highest concentrations of 2,6-dimethoxyphenol were present on Day 7 for both male and female groups (25.9 and 76.5 ng/mL, respectively).

For oral gavage dosing, based on post-dosing observations (including one male animal at 1,400 mg/kg bw per day that was euthanised due to morbidity) and decreases in animal body weights, the estimated MTDs for an extended dosing regimen of 28-days duration was 700 mg/kg bw per day for males and 1,400 mg/kg bw per day for females.

For the dietary route, based on body weight loss observed in one male at 35,000 mg/kg diet after 15 days of exposure, the estimated maximum tolerated concentration for males was considered to be 25,000 mg/kg diet for a 28-day dosing period. In females, based on an absence of changes attributed to the test item, 50,000 mg/kg diet was considered to be the maximum feasible concentration, in order to avoid nutritional disturbances.

The dietary route of administration was chosen for the main study. Since sex-related differences in the MTD were observed, both male and female transgenic mice were used in the main study.

In the *in vivo* gene mutation assay in Muta™Mouse (*lacZ/GalE*), Zesti Smoke Code 10 (batch no. 05210465) was administered via the diet to three groups of male and three groups of female transgenic $CD_2$-LacZ80/HazfBR mice (seven animals per group) for 28 consecutive days (Labcorp, 2022b). The study was performed according to OECD TG 488 (OECD, 2020b) and in compliance with GLP. Male groups received dietary concentrations of 6,250, 12,500 or 25,000 mg/kg diet and female groups received dietary concentrations of 12,500, 25,000 or 50,000 mg/kg diet. The concentrations corresponded to dose levels of 1,024, 2,011 and 4,206 mg/kg bw per day for males and 2,491, 4,894 and 10,284 mg/kg bw per day for females. 5 KB3 (5LF2 EU) Rodent Diet was used as the vehicle control (12 animals per group). The treatment period was followed by a 3-day manifestation period and then animals were sacrificed, and the liver and glandular stomach removed. The positive control used samples from animals (five/six animals per group) administered ethyl-N-nitrosourea (ENU) at a dose of 50 mg/kg bw from a previous study.

Separate satellite groups of Muta™Mice (seven males or six females per group) were also included within the study design in order to obtain plasma and tissue samples from the liver and glandular stomach at the end of the 28-day dosing period. In these tissues the marker analyte 2,6-dimethoxyphenol was detected.

In all groups receiving the test item, there were no mortalities, no clinical signs of toxicity, no differences in body weight gains (with the exception of one male in the top group which lost 2.4% body weight by day 28), organ weights (liver and glandular stomach) or food consumption.

Liver and glandular stomach samples from at least five animals per dose groups and positive control groups and at least six animals from the vehicle control groups were processed for DNA isolation.

For each DNA sample from vehicle control or test item-treated animals, the number of plaques from three to six packagings was greater than 200,000 (i.e. more than the OECD recommended minimum of 125,000 plaques). In the positive controls, there were four instances where data from one to two packaging(s) yielded plaque numbers below the OECD-specified minimum of 125,000 plaques per tissue per animal.

The mean vehicle control mutant frequency data for the liver and glandular stomach fell within the laboratory's respective historical ranges (animal sex not specified). For individual animal results falling outside the respective control ranges, there were three incidences in female liver, four incidences in male glandular stomach and two incidences in female glandular stomach. However, the study author considered that the values were similar enough to the respective historical control ranges to consider the data acceptable. The positive controls gave elevated mutant frequencies compared to the vehicle controls.

In females, treatment with Zesti Smoke Code 10 did not significantly increase the mean mutation frequency at the *lacZ* gene in the liver at the lower doses, though the mutation frequency for three animals (22.61 and 21.75 $\times$ $10^6$ plaque forming units (pfu) from the 12,500 mg/kg diet group and 11.82 $\times$ $10^6$ pfu from the 25,000 group) fell below the test laboratory's historical vehicle 95% reference range (22.63–117.97 $\times$ $10^6$ pfu) and in one case also below the historical vehicle control observed range (15.65–382.28 $\times$ $10^6$ pfu). At the top dose, all individual animal and group mean mutation frequency values fell within the historical vehicle control 95% reference range. However, there was a small, but statistically significant increase ($p \leq 0.05$) in the group mean mutation frequency (48.11 $\times$ $10^6$ pfu) compared to vehicle control (21.65 $\times$ $10^6$ pfu) which contributed to a statistically significant linear trend ($p \leq 0.01$). This increase was primarily attributed to one animal within the group demonstrating an elevated mutation frequency value (95.57 $\times$ $10^6$ pfu). The study authors considered that the increases in mutation frequency were within the normal variation for the assay and that the statistical significance was of no biological relevance.

In males, treatment with Zesti Smoke Code 10 did not significantly increase the mutation frequency at the *lacZ* gene in the liver as all individual and mean mutation frequencies fell within the historical vehicle control 95% reference range, with the exception of two animals from the low dose group (13.61 and 16.72 $\times$ $10^6$ pfu) that fell below this range, one of which also fell below the historical vehicle control observed range. There was no dose–response relationship.

In the glandular stomach, no statistically significant increases in mutation frequency were observed in males or females, with most individual animal and all group mean mutation frequencies falling within the laboratory's historical vehicle control observed range (29.04–119.50 $\times$ $10^6$ pfu, based on only 25 animals). There were exceptions for individual animals from all groups, except for the mid-dose group for males, which fell below the laboratory's historical vehicle control observed range. For the treated females, there was no evidence of a dose-related response. For the treated males, an increase in the group mean mutation frequency for the intermediate dose group (12,500 mg/kg diet) contributed to a statistically significant linear trend ($p \leq 0.05$). However, as all individual animal and group mean mutation frequencies fell within the laboratory's historical vehicle control observed range, and with the exception of data for one animal all individual data fell below the historical vehicle control group mean, the study authors considered the trend to be of no biological relevance.

The study authors concluded that in this *in vivo* gene mutation assay in Muta™Mouse (*lacZ/galE*), Zesti Smoke Code 10 did not induce a statistically significant increase in mutation frequency in the liver or glandular stomach under the conditions of the study.

Irrespective of the analytical results for the plasma analysis 2,6-dimethoxyphenol, since the study focussed on stomach and liver (site of contact tissues), the Panel agreed with this conclusion from the study authors and considered the study to be reliable without restrictions and the results of high relevance.

Results of *in vivo* studies are summarised in Appendix E, Table E.2.

# 4. Discussion

The European Commission has requested the European Food Safety Authority (EFSA) to evaluate the safety of the smoke flavouring Primary Product Zesti Smoke Code 10 (SF-002), for which a renewal application has been submitted, in accordance with Article 12(1) of Regulation (EC) No 2065/2003.

The Primary Product is produced from hardwood sawdust comprising 50–60% hickory (*C. ovata*) and 40–50% white oak (*Q. alba*).

The Panel considered the information provided on the manufacturing process as sufficient. The data demonstrated that the Primary Product is produced in the same way as the product evaluated formerly (EFSA CEF Panel, 2009).

The applicant provided compositional data for only one batch of the Primary Product. The water content was the only compositional parameter for which data from three replicates were provided. The Panel recognises that this creates a non-standard uncertainty with respect to the reproducibility, however the Panel had sufficient confidence to use the compositional data (water content and wt% of identified and quantified volatiles) to perform the safety assessment of the Primary Product.

Despite the limitations in the quantification of the volatile constituents, the Panel concluded that the applied method meets the legal quality criterion that at least 50% by mass of the solvent-free fraction shall be identified and quantified (Regulation (EC) No 627/2006)).

Regarding the identified and quantified proportion of the volatile fraction, given the shortcomings in the quantitative data submitted by the applicant (see Section 3.1.2.4.1), the Panel could not judge whether the applied method meets the legal quality criterion that at least 80% of the volatile fraction shall be identified and quantified (Regulation (EC) No 627/2006).

The applicant reported data on the batch-to-batch variability of 441 batches of the Primary Product. The observed relative standard deviations for the monitored chemical parameters were on average < 9% (see Table 6). In addition, the applicant performed statistical analyses for 18 selected compounds in 26 batches produced from July 2020 to January 2022. The observed relative standard deviations were on average < 16%. The data provided demonstrated that the batch-to-batch variability of the Primary Product was sufficiently low. The Panel noted that the applicant has adequate control over the relevant steps of the production process (pyrolysis and purifications) and concluded that the data provided on the selected batches are representative of the Primary Product.

With respect to stability over time, the applicant provided data on chemical classes of the Primary Product. In particular, the concentrations of phenols and carbonyls showed significant decreases over a storage period of 2 years; no information on possible degradation/reaction products was provided. The applicant did not provide stability data based on the analysis of individual constituents. These data gaps create a non-standard uncertainty with respect to the stability of the Primary Product. Given this uncertainty, the Panel could not judge whether the shelf-life of 2 years, as reported by the applicant, for the Primary Product is appropriate.

Based on the data provided, the Panel considered that the evidence is not conclusive to exclude the presence of small particles including nanoparticles from the Primary Product. If based on additional evidence, the presence of small particles including nanoparticles cannot be eventually excluded, a specific assessment at the nanoscale would be required, in line with the EFSA Scientific Committee Guidance on risk assessment of nanomaterials (EFSA Scientific Committee, 2021).

The applicant proposed limits for four toxic elements (arsenic, cadmium, lead and mercury), which are lower than in the current EU specifications (Table 7). The Panel noted that the proposed limits are in line with the reported levels of toxic elements in the commercial samples of the Primary Product, apart from cadmium for which the proposed specification limit is lower than the highest measured value of 0.04 mg/kg (see Table 3).

The Panel performed a risk assessment on the presence of these toxic elements in the Primary Product and concluded that, when considering the current limits of the EU specifications (scenario (i) in Table 14), the ranges of the calculated MOE values for arsenic were insufficient, i.e. below the target value of 1,000. For the other three toxic elements (cadmium, lead and mercury), their presence in the Primary Product up to the current limits in the EU specifications does not give rise to a safety concern. When considering the highest measured values for Cd multiplied by a factor of 5 and the LOQs multiplied by a factor of 10 for As, Hg and Pb (scenario (ii) in Table 14), the Panel concluded for arsenic that (a) the lower end of the range for the highest mean and (b) the range for the highest 95th percentile of the calculated MOE values were insufficient, i.e. below the target value of 1,000. In this scenario, the presence of the other toxic elements in the Primary Product do not give rise to concern.

The analytical procedure for the determination of 16 PAHs meets the performance criteria as set in Regulation (EC) No 627/2006. The levels of benzo[a]pyrene and benzo[a]anthracene were below the current limits in Regulation (EC) No 2065/2003. Based on the estimated exposure to the Primary Product and the maximum reported level of the PAH4 (i.e. the sum of their LOQs) in the Primary Product (i.e. 2.0 μg/kg), an MOE of at least $1.3 \times 10^7$ could be calculated for the exposure to PAHs, which would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions (see EFSA Scientific Committee, 2012). The Panel noted that including a limit for PAH4 in the EU specifications would take better account of the presence of other PAHs than only the two PAHs benzo[a]pyrene and benzo[a]anthracene.

Overall, the Panel considered that limits in the EU specifications for the four toxic elements and PAH4 should be established based on actual levels in the commercial Primary Product. If the European Commission decides to revise the limits already present and to include a limit for PAH4, the estimated exposure to the four toxic elements and PAH4 as presented in Sections 3.3.3.1 and 3.3.3.2 could be considered.

The Primary Product is requested to be authorised for use in 13 food categories. The Panel performed an exposure assessment for this product based on proposed maximum and expected typical use levels in these food categories, using both FAIM and DietEx. In general, the use of FAIM or DietEx results in an overestimation of the exposure. However, this overestimation is less pronounced (i.e. less conservative) using DietEx than using FAIM for this Primary Product, because DietEx allows a better selection of the actual foods to which the Primary Product may be added. Therefore, the DietEx exposure estimates have been used for the risk assessment of the Primary Product.

At the maximum proposed use levels, mean DietEx exposure estimates to the Primary Product from its use as a smoke flavouring ranged from 0.02 mg/kg bw per day in infants to 4.6 mg/kg bw per day in toddlers (Table 12). The 95th percentiles DietEx exposure estimates ranged from no dietary exposure in infants to 13.0 mg/kg bw per day in toddlers. At the expected typical use levels, the mean DietEx dietary exposure estimates ranged from 0.01 mg/kg bw per day in infants to 1.8 mg/kg bw per day in toddlers, and the 95th percentile DietEx exposure estimates ranged from no dietary exposure in infants to 5.2 mg/kg bw per day in toddlers (Table 12).

Regarding the genotoxicity data, the Panel conducted the evaluation in line with the currently applicable EFSA guidance on smoke flavourings (EFSA FAF Panel, 2021) which encompasses all the EFSA guidance documents on genotoxicity (EFSA Scientific Committee, 2011, 2017b, 2019, 2021b).

From the analysis of the available information on genotoxicity of the 50 individual components of the Primary Product, the Panel considered that:

i) for 44 individual components no concern for genotoxicity is identified (see Annex B);
ii) a concern for genotoxicity is identified for two components, i.e. furan-2(5*H*)-one and benzene-1,2-diol, which are both present in the Primary Product at average concentrations of 0.3 wt%;
iii) for 4 components a potential concern for genotoxicity is identified, for which additional data would be needed to reach a conclusion on the genotoxic potential of these substances.

The details of the genotoxicity data available on the six components listed in (ii) and (iii) are given and discussed in Appendix B.

Regarding the two components furan-2(5*H*)-one and benzene-1,2-diol, the available data raise a concern for genotoxicity. As described in Appendix B, the Panel considered that furan-2(5*H*)-one displays a genotoxic activity *in vivo*, observed in a Comet assay in liver (EFSA FAF Panel, 2019) and in a MN assay in liver (EFSA FAF Panel, 2023a, 2023b, 2023c). From these EFSA opinions, no evidence is available to prove that furan-2(5*H*)-one induces chromosomal damage via a threshold-based mechanism. Therefore, the Panel considered that the derivation of reference points from the available genotoxicity studies and the calculation of a MOE, as proposed by the applicant, is not appropriate.

Regarding benzene-1,2-diol, the Panel considered the evaluation of the ECHA's Risk Assessment Committee (ECHA, 2016a) and agreed that based on experimental *in vitro* and *in vivo* data (including studies where animals were exposed via oral route) a concern for genotoxicity *in vivo* is identified.

The Panel investigated if the concern for genotoxicity for furan-2(5*H*)-one and benzene-1,2-diol and the potential concern for genotoxicity for the four components mentioned above in (iii) could be ruled out by application of the TTC approach for DNA-reactive mutagens and/or carcinogens (EFSA Scientific Committee, 2019). The obtained exposure estimates were compared with the TTC value of 0.0025 μg/kg bw per day for DNA-reactive mutagens and/or carcinogens. For five substances, the exposure

estimates were well above this TTC value (see Table 16) and therefore the application of the TTC approach could not rule out the (potential) concern for genotoxicity for these components. The comparison with the TTC was not possible for 2-propenal (acrolein), because its concentration in the Primary Product was not determined.

The Panel considered whether refined exposure estimates for the Primary Product (in line with the principles described in the guidance on smoke flavourings (EFSA FAF Panel, 2021)) could mitigate the concern for the (potential) genotoxicity of each of the five components that have been quantified in the Primary Product.

However, taking into account:

- the magnitude of the calculated ratios between the exposure estimates and the above-mentioned TTC value (see Table 16);
- the uses of the Primary Product and the nature of the restrictions/exceptions indicated by the applicant for the different food categories (see Table 9),

the Panel considered that a more refined exposure assessment will not reduce the exposure estimates for these components to such an extent that they will be below the TTC value of 0.0025 μg/kg bw per day.

The Primary Product (whole mixture) was tested in *in vitro* and *in vivo* genotoxicity studies to investigate the genotoxicity of the unidentified fraction of the Primary Product, in line with the EFSA Scientific Committee statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019).

The Primary Product, *in vitro*, induced gene mutations in two bacterial reverse mutation studies and in mammalian cells. However, *in vivo*, it did not induce gene mutations in liver and glandular stomach of transgenic mice.

The Primary Product induced chromosomal damage *in vitro* based on results from a chromosomal aberration test and structural or numerical chromosomal aberrations based on the results from a MN test in human peripheral blood lymphocytes.

The Primary Product was tested in two *in vivo* MN studies in bone marrow. In both studies no increase in micronucleated cells was observed, but these studies were evaluated as of low relevance due to insufficient demonstration of bone marrow exposure.

Since the results of the *in vivo* MN studies are of low relevance, they are not sufficient to rule out the concern for genotoxicity for the whole mixture that is raised by the observation of chromosomal aberrations in *in vitro* assays.

In principle, based on the EFSA Scientific Committee statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019) as well as on the EFSA guidance on smoke flavourings (EFSA FAF Panel, 2021), if aneugenicity can be excluded, an *in vivo* Comet assay (OECD TG 489 (2016d)) at the site of contact and in the liver might also be considered appropriate to follow-up the chromosomal aberrations observed *in vitro*. The studies at the site of contact allow investigation of genotoxic effects at the site where the exposure to the components is expected to be maximal. However, in this case, the concern for genotoxicity for the Primary Product cannot be ruled out by an additional *in vivo* Comet assay performed on the whole mixture, since the exposure estimate for the two genotoxic components furan-2(5*H*)-one and benzene-1,2-diol are both above the TTC value of 0.0025 μg/kg bw per day for DNA-reactive mutagens and/or carcinogens. In fact, as outlined in the EFSA Scientific Committee statement on genotoxicity assessment on chemical mixtures (EFSA Scientific Committee, 2019), 'if the mixture contains one or more chemical substances that are evaluated to be genotoxic *in vivo* via a relevant route of administration, the whole mixture raises concern about genotoxicity'.

For the same reason, the Panel noted that, filling of the data gaps for the four components that raise a potential concern for genotoxicity, as pointed out in Appendix B, will not remove the safety concern for the Primary Product.

## 5. Conclusions

In line with the ToR as provided by the European Commission, in the current opinion EFSA assessed the chemical characterisation, the genotoxicity and the dietary exposure to Zesti Smoke Code 10 (SF-002).

From all data available on characterisation, the Panel concluded that the Primary Product considered in this opinion is representative for the one authorised in Commission Implementing

Regulation (EU) No 1321/2013 under the code name SF-002. Nevertheless, the Panel concluded that the compositional data provided on the Primary Product were not adequate. The size of the unidentified volatile fraction could only be roughly estimated. Therefore, the Panel could not judge whether the applied methods meet the legal quality criterion that at least 80% by mass of the volatile fraction shall be identified and quantified, as set in Regulation (EC) No 627/2006. The Panel concluded that the applicant has adequate control over the production process but considering the limited data provided on the stability of the Primary Product, the Panel could not judge whether the shelf-life of 2 years, as reported by the applicant, is appropriate.

Since the Primary Product contains an unidentified fraction that has not been characterised in terms of solubility and particle size, the Panel could not exclude the presence of small particles including nanoparticles and hence could not conclude if a conventional risk assessment is sufficient or whether it needs to be complemented with nano-specific considerations.

The Panel identified a potential concern for genotoxicity for four components in the Primary Product as well as for the unidentified fraction of the mixture. More importantly, the Primary Product contains furan-2(5*H*)-one and benzene-1,2-diol, two known *in vivo* genotoxic substances via the oral route. Considering that the exposure estimates for furan-2(5*H*)-one and benzene-1,2-diol are above the TTC of 0.0025 μg/kg bw per day (or 0.15 μg/person per day) for DNA-reactive mutagens and/or carcinogens, the Panel concluded that Zesti Smoke Code 10 (SF-002) raises concern with respect to genotoxicity.

## 6. Documentation as provided to EFSA


1) Dossier "Application for renewal of an already authorised smoke flavouring – Zesti Smoke Code 10". Dossier number: SFL-2022-7010. June 2022. Submitted by Kerry Inc.[19]
2) Additional data received on 10 February 2023, submitted by Kerry Inc. in response to additional data request from EFSA sent on 24 November 2022.
3) Additional data received on 22 June 2023 submitted by Kerry Inc. as spontaneous submissions.
4) Covance, 2004. Flavouring 37735: reverse mutation in five histidine-requiring strains of Salmonella typhimurium. Covance Laboratories Ltd. Study No. 2330/1 May 2004. Unpublished study report submitted by Kerry Inc.
5) Covance, 2005. Flavouring 37735: measurement of unscheduled DNA synthesis in rat liver using an *in vivo*/*in vitro* procedure. Covance Laboratories Ltd. Study No. 2330/7 September 2005. Unpublished study report submitted by Kerry Inc.
6) Covance, 2006a. Flavouring 37735: mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre$^{R}$ Fluctuation technique. Covance Laboratories Ltd. Study No. 2330/3 January 2006. Unpublished study report submitted by Kerry Inc.
7) Covance, 2006b. Flavouring 37735: induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Covance Laboratories Ltd. Study No. 2330/2 January 2006. Unpublished study report submitted by Kerry Inc.
8) Covance, 2006c. Flavouring 37735: induction of micronuclei in the bone marrow of treated rats. Covance Laboratories Ltd. Study No. 2330/8 January 2006. Unpublished study report submitted by Kerry Inc.
9) Labcorp, 2021a. Code 10 (SF-002): Bacterial Reverse Mutation Assay. Labcorp Early Development Laboratories Ltd. Study No. 8447529, September 2021. Unpublished study report submitted by Kerry Inc.
10) Labcorp, 2021b. Code 10 (SF-002): *In Vitro* Human Lymphocyte Micronucleus Assay. Labcorp Early Development Laboratories Ltd. Study No. 8447530, September 2021. Unpublished study report submitted by Kerry Inc.
11) Labcorp, 2021c. Code 10 (SF-002) [Item code 707433]: Validation of an Analytical Method and Formulation Accuracy, Homogeneity and Stability in Liquid Vehicle and Diet. Labcorp Early Development Laboratories Ltd. Study No. 8447534, August 2021. Unpublished study report submitted by Kerry Inc.


[19] The non-confidential version of the dossier, following EFSA's assessment of the applicant's confidentiality requests, is published on Open.EFSA and is available at the following link: https://open.efsa.europa.eu/dossier/SFL-2022-7010

12) Labcorp, 2022a. Code 10 (SF-002): Rat Bone Marrow Micronucleus Assay. Labcorp Early Development Laboratories Ltd. Study No. 8447532, May 2022. Unpublished study report submitted by Kerry Inc.
13) Labcorp, 2022b. Code 10 (SF-002): Code 10 (SF-002): Transgenic Gene Mutation Assay in Muta™Mice. Labcorp Early Development Laboratories Ltd. Study No. 8476534, August 2022. Unpublished study report submitted by Kerry Inc.
14) Labcorp, 2022c. Code 10 (SF-002): Code 10 (SF-002): Exploratory 14-Day Dose Range-Finding Study in Muta™Mice. Labcorp Early Development Laboratories Ltd. Study No. 8447533, March 2022. Unpublished study report submitted by Kerry Inc.

## References


Aydın B, Şekeroğlu AZ and Şekeroğlu V, 2018. Effects of whey protein and conjugated linoleic acid on acrolein-induced cardiac oxidative stress, mitochondrial dysfunction, and dyslipidemia in rats. Biomedicine and Pharmacotherapy, 107, 901–907. https://doi.org/10.1016/j.biopha.2018.08.081

Benigni R, Bossa C, Jeliazkova N, Netzeva T and Worth A, 2008. The Benigni/Bossa rulebase for mutagenicity and carcinogenicity - a module of Toxtree. JRC Scientific and Technical, Reports, OPOCE, Luxembourg. 70 p. Available online: https://publications.jrc.ec.europa.eu/repository/handle/JRC43157

Benigni R, Bossa C, Tcheremenskaia O and Worth A, 2009. Development of Structural Alerts for the In vivo Micronucleus Assay in Rodents. JRC Scientific and Technical Reports, OPOCE, Luxembourg. pp. 42 Available online: https://publications.jrc.ec.europa.eu/repository/handle/JRC52274

BfR (Bundesinstitut für Risikobewertung), 2006. Assessment of the carcinogenicity of formaldehyde [CAS No. 50–00-0]. 156 pp. ISBN 3–938163–14-3 Available online: https://mobil.bfr.bund.de/cm/350/assessment_of_the_carcinogenicity_of_formaldehyde.pdf

Cai Y, Wu MH, Ludeman SM, Grdina DJ and Dolan ME, 1999. Role of $O^6$-Alkylguanine-DNA Alkyltransferase in Protecting against Cyclophosphamide-induced Toxicity and Mutagenicity. Cancer Research, 59, 3059–3063.

Culp SJ, Gaylor DW, Sheldon WG, Goldstein LS and Beland FA, 1998. A comparison of the tumours induced by coal tar and benzo[a]pyrene in a 2-year bioassay. Carcinogenesis, 19, 117–124. https://doi.org/10.1093/carcin/19.1.117

Curren RD, Yang LL, Conklin PM, Grafstrom RC and Harris CC, 1988. Mutagenesis of xeroderma pigmentosum fibroblasts by acrolein. Mutation Research, 209, 17–22. https://doi.org/10.1016/0165-7992(88)90104-2

Demir E, Kaya B, Soriano C, Creus A and Marcos R, 2011. Genotoxic analysis of four lipid-peroxidation products in the mouse lymphoma assay. Mutation Research, 726, 98–103. https://doi.org/10.1016/j.mrgentox.2011.07.001

Denkel E, Pool BL, Schlehofer JR and Eisenbrand G, 1986. Biological activity of N-nitrosodiethanolamine and of potential metabolites which may arise after activation by alcohol dehydrogenase in Salmonella typhimurium, in mammalian cells, and in vivo. Journal of Cancer Research Clinical Oncology, 111, 149–153. https://doi.org/10.1007/bf00400754

ECHA (European Chemicals Agency), 2008. Guidance for the implementation of REACH, Chapter R.6: QSARs and grouping of chemicals. ECHA, Helsinki. 134 pp. Available online: https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

ECHA (European Chemicals Agency), 2011. Guidance on information requirements and chemical safety assessment. Chapter R.4: Evaluation of available information Available online: https://echa.europa.eu/documents/10162/13643/information_requirements_r4_en.pdf/d6395ad2-1596-4708-ba86-0136686d205e

ECHA (European Chemicals Agency), 2012. Opinion of the committee for risk assessment on the proposal for the harmonised classification and labelling at EU level; Formaldehyde. Available online: https://echa.europa.eu/documents/10162/b8dfa022-9544-72e8-dcaa-7491dff3c0d5

ECHA (European Chemicals Agency), 2016a. Opinion of the committee for risk assessment on the proposal for the harmonised classification and labelling at EU level; 1,2-dihydroxybenzene; pyrocatechol Available online: https://echa.europa.eu/documents/10162/3cb7d6c4-3bee-39cc-ca20-e52aa6565301

ECHA (European Chemicals Agency), 2016b. Opinion of the committee for risk assessment on the proposal for the harmonised classification and labelling at EU level; acetaldehyde; ethanal. Available online: https://echa.europa.eu/documents/10162/0dae8db6-8f8c-428d-b591-7e5f04fc6b08

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2018. Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure. Available online: https://zenodo.org/records/1256085

EFSA AFC Panel (EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food), 2007. Opinion on the use of formaldehyde as a preservative during the manufacture and preparation of food additives. EFSA Journal 2007;5(1):415, 10 pp. https://doi.org/10.2903/j.efsa.2007.415

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2009. Scientific Opinion on the safety of smoke flavour Primary Product – Zesti Smoke Code 10. EFSA Journal 2009;7(6):982, 24 pp. https://doi.org/10.2903/j.efsa.2009.982

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific Opinion on the Safety of smoke flavour Primary Product Zesti Smoke Code 10-2011 Update. EFSA Journal 2011;9(7):2307, 17 pp. https://doi.org/10.2903/j.efsa.2011.2307
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2008. Scientific opinion on Polycyclic Aromatic Hydrocarbons in Food. EFSA Journal 2008;6(8):724, 114 pp. https://doi.org/10.2903/j.efsa.2008.724
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009a. Scientific Opinion on Arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009b. Scientific Opinion on Cadmium in food. EFSA Journal 2009;7(3):980, 139 pp. https://doi.org/10.2903/j.efsa.2009.980
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010. Scientific Opinion on Lead in food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985
EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2019. Scientific Opinion on Flavouring Group Evaluation 217, Revision 2 (FGE.217Rev2), Consideration of genotoxic potential for α,β-unsaturated ketones and precursors from chemical subgroup 4.1 of FGE.19: lactones. EFSA Journal 2019;17(1):5568, 45 pp. https://doi.org/10.2903/j.efsa.2019.5568
EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2021. Scientific Guidance for the preparation of applications on smoke flavouring Primary Products. EFSA Journal 2021;19(3):6435, 40 pp. https://doi.org/10.2903/j.efsa.2021.6435
EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2023a. Scientific opinion on the renewal of the authorisation of proFagus Smoke R714 (SF-001) as a smoke flavouring Primary Product. EFSA Journal 2023;21(11):8363, 55 pp. https://doi.org/10.2903/j.efsa.2023.8363
EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2023b. Scientific opinion on the renewal of the authorisation of Smoke Concentrate 809045 (SF-003) as a smoke flavouring Primary Product. EFSA Journal 2023;21(11):8365. https://doi.org/10.2903/j.efsa.2023.8365
EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2023c. Scientific opinion on the renewal of the authorisation of proFagus Smoke R709 (SF-008) as a smoke flavouring Primary Product. EFSA Journal 2023;21(11):8369. https://doi.org/10.2903/j.efsa.2023.8369
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014a. Scientific Opinion on the safety and efficacy of formaldehyde for all animal species based on a dossier submitted by Adiveter S.L. EFSA Journal 2014;12(2):3562, 25 pp. https://doi.org/10.2903/j.efsa.2014.3562
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014b. Scientific Opinion on the safety and efficacy of formaldehyde for all animal species based on a dossier submitted by Regal BV. EFSA Journal 2014;12(2):3561, 24 pp. https://doi.org/10.2903/j.efsa.2014.3561
EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessment carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(4):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051
EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379
EFSA Scientific Committee, 2012. Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA Journal 2012;10(3):2578, 5 pp. https://doi.org/10.2903/j.efsa.2012.2578
EFSA Scientific Committee, 2017a. Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. https://doi.org/10.2903/j.efsa.2017.4658
EFSA Scientific Committee, 2017b. 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
EFSA Scientific Committee, 2019. Statement on the genotoxicity assessment of chemical mixtures. EFSA Journal 2019;17(1):5519, 11 pp. https://doi.org/10.2903/j.efsa.2019.5519
EFSA Scientific Committee, 2021a. Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles. EFSA Journal 2021;19(8):6769, 48 pp. https://doi.org/10.2903/j.efsa.2021.6769
EFSA Scientific Committee, 2021b. Scientific Opinion on the guidance on aneugenicity assessment. EFSA Journal 2021;19(8):6770, 27 pp. https://doi.org/10.2903/j.efsa.2021.6770
Epstein SS, Arnold E, Andrea J, Bass W and Bishop Y, 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicology and Applied Pharmacollogy, 23, 288–325. https://doi.org/10.1016/0041-008x(72)90192-5
Foiles PG, Akerkar SA, Miglietta LM and Chung F-L, 1990. Formation of cyclic deoxyguanosine adducts in Chinese hamster ovary cells by acrolein and crotonaldehyde. Carcinogenesis, 11, 2059–2061. https://doi.org/10.1093/carcin/11.11.2059

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick MA, Anderson B and Zeiger E, 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environmental and Molecular Mutagenesis, 10(S10), 1–35.

Gardner R, Kazi S and Ellis EM, 2004. Detoxication of the environmental pollutant acrolein by a rat liver aldo-keto reductase. Toxicology Letters, 148, 65–72. https://doi.org/10.1016/j.toxlet.2003.12.056

Garst J, Stapleton P and Johnston J, 1983. Mutagenicity of alphahydroxy ketones may involve superoxide anion radical. Oxy radicals and their scavenger systems. Cellular and Medical Aspects, 2, 125–130.

HCN (Health Council of the Netherlands), 2011. 1,2-Catechol (pyrocatechol); Evaluation of the carcinogenicity and genotoxicity. Publication no. 2011/05OSH. ISBN: 978–90–5549-829-1. Available online: https://www.healthcouncil.nl/documents/advisory-reports/2011/02/25/1-2-catechol-pyrocatechol-evaluation-of-the-carcinogenicity-and-genotoxicity

Hengstler JG, Fuchs J, Gebhard S and Oesch F, 1994. Glycolaldehyde causes DNA-protein crosslinks: a new aspect of ethylene oxide genotoxicity. Mutation Research, 304, 229–234. https://doi.org/10.1016/0027-5107(94)90215-1

IARC (International Agency for Research on Cancer), 1999. Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. Catechol. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 71 Available online: https://www.ncbi.nlm.nih.gov/books/NBK498783/

IARC (International Agency for Research on Cancer), 2006. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 88 Available online: https://publications.iarc.fr/_publications/media/download/2757/c6a9fc1529ff03f196d3595caf6e198cb9b104ce.pdf

IARC (International Agency for Research on Cancer), 2012a. Personal habits and indoor combustions. IARC Monographs on the evaluation of carcinogenic risks to humans Volume 100E Available online: https://publications.iarc.fr/_publications/media/download/5255/acb03faa0bbde7cf84987d1d7d287e2f74e8399b.pdf

IARC (International Agency for Research on Cancer), 2012b. Chemical Agents and Related Occupations. IARC monographs on the evaluation of carcinogenic risks to humans Volume 100F Available online: https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Chemical-Agents-And-Related-Occupations-2012

IARC (International Agency for Research on Cancer), 2021. Acrolein, Crotonaldehyde, and Arecoline. IARC Monographs on the Identification of Carcinogenic Hazards to Humans Volume 128 Available online: https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Acrolein-Crotonaldehyde-And-Arecoline-2021

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series no. 884 Available online: https://apps.who.int/iris/bitstream/handle/10665/42142/WHO_TRS_884.pdf;jsessionid=A63B9D3D64D875629D0441AF217876AE?sequence=1v

Johnson GE, Soeteman-Hernández LG, Gollapudi BB, Bodger OG, Dearfield KL, Heflich RH, Hixon JG, Lovell DP, MacGregor JT, Pottenger LH, Thompson CM, Abraham L, Thybaud V, Tanir JY, Zeiger E, van Benthem J and White PA, 2014. Derivation of point of departure (PoD) estimates in genetic toxicology studies and their potential applications in risk assessment. Environmental and Molecular Mutagenesis, 55, 609–623. https://doi.org/10.1002/em.21870

Kim SI, Pfeifer GP and Besaratinia A, 2007. Lack of mutagenicity of acrolein-induced dna adducts in mouse and human cells. Cancer Research, 67, 11640–11647. https://doi.org/10.1158/0008-5472.CAN-07-2528

Klimisch HJ, Andreae M and Tillmann U, 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology, 25, 1–5. https://doi.org/10.1006/rtph.1996.1076

Menz J, Götz ME, Gündel U, Gürtler R, Herrmann K, Hessel-Pras S, Kneuer C, Kolrep F, Nitzsche D, Pabel U, Sachse B, Schmeisser S, Schumacher DM, Schwerdtle T, Tralau T, Zellmer S and Schäfer B, 2023. Genotoxicity assessment: opportunities, challenges and perspectives for quantitative evaluations of dose–response data. Archives Toxicology, 97, 2303–2328. https://doi.org/10.1007/s00204-023-03553-w

Migliore L, Ventura L, Barale R, Loprieno N, Castellino S and Pulci R, 1989. Micronuclei and nuclear anomalies induced in the gastro-intestianl epithelium of rats treated with formaldehyde. Mutagenesis, 4, 327–334. https://doi.org/10.1093/mutage/4.5.327

NTP (National Technology Program), 2006. Technical Report on the comparative toxicity studies of allyl acetate (CAS No. 591–87-7), allyl alcohol (CAS No. 107–18-6) and acrolein (CAS No. 107–02-8) administered by gavage to F344/N rats and B6C3F1 mice. Toxic Rep Ser., 48, pp. 1–73, A1–H10. PMID: 17160105.

OECD (Organisation for Economic Co-operation and Development), 1995. Test No. 105: Water Solubility. OECD Guidelines for the Testing of Chemicals, Section 1, OECD Publishing, Paris, 7 pp. https://doi.org/10.1787/9789264069589-en

OECD (Organisation for Economic Co-operation and Development), 1997. Test No. 474: Mammalian Erythrocyte Micronucleus Test. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, 10 pp. https://doi.org/10.1787/9789264071285-en

OECD (Organisation for Economic Co-operation and Development), 2003. Detailed review paper on cell transformation assays for detection of chemical carcinogens. OECD Series on Testing and Assessment No. 31. Available online: https://www.oecd.org/chemicalsafety/testing/37863750.pdf
OECD (Organisation for Economic Co-operation and Development), 2016a Test No. 490: In vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, 21 pp. https://doi.org/10.1787/9789264264908-en
OECD (Organisation for Economic Co-operation and Development), 2016b. Test No. 473: In vitro Mammalian Chromosomal Aberration Test. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, 22 pp. https://doi.org/10.1787/9789264264649-en
OECD (Organisation for Economic Co-operation and Development), 2016c. Test No. 474: Mammalian Erythrocyte Micronucleus Test. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, 21 pp. https://doi.org/10.1787/9789264264762-en
OECD (Organisation for Economic Co-operation and Development), 2016d. Test No. 489: In Vivo Mammalian Alkaline Comet Assay. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. pp. 27. https://doi.org/10.1787/9789264264885-en
OECD (Organisation for Economic Co-operation and Development), 2016e. Test No. 487: In Vitro Mammalian Cell Micronucleus Test. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. pp. 29. https://doi.org/10.1787/9789264264861-en
OECD (Organisation for Economic Co-operation and Development), 2020a. Test No. 471: Bacterial Reverse Mutation Test. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. pp. 12. https://doi.org/10.1787/9789264071247-en
OECD (Organisation for Economic Co-operation and Development), 2020b. Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. pp. 29. https://doi.org/10.1787/9789264203907-en
Smith RA, Williamson DS, Cerny RL and Cohen SM, 1990. Detection of 1,N6-propanodeoxyadenosine in acrolein-modified polydeoxyadenylic acid and DNA by 32P postlabeling. Cancer Research, 50, 3005–3012.
Whitwell J, Smith R, Chirom T, Watters G, Hargreaves V, Lloyd M, Phillips S and Clements J, 2019. Inclusion of an extended treatment with recovery improves the results for the human peripheral blood lymphocyte micronucleus assay. Mutagenesis, 34, 217–237. https://doi.org/10.1093/mutage/gez011
WHO (World Health Organization), 2002. Formaldehyde. Concise international chemical assessment document, 40, 81 pp. Available online: https://iris.who.int/bitstream/handle/10665/42430/a73769.pdf?sequence=1&isAllowed=y
Yamaguchi T, 1982. Mutagenicity of Trioses and Methyl Glyoxal on Salmonella typhimurium. Agricultural and Biological Chemistry, 46, 849–851. https://doi.org/10.1080/00021369.1982.10865161
Yamaguchi T and Nakagawa K, 1983. Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. Agricultural and Biological Chemistry, 47, 2461–2465. https://doi.org/10.1080/00021369.1983.10865979
Zhang S, Chen H, Wang A, Liu Y, Hou H and Hu Q, 2018. Combined effects of co-exposure to formaldehyde and acrolein mixtures on cytotoxicity and genotoxicity in vitro. Environmental Science Pollution Research, 25, 25306–25314. https://doi.org/10.1007/s11356-018-2584-z
Zhang S, Chen H, Zhang J, Li J, Hou H and Hu Q, 2020. The multiplex interactions and molecular mechanism on genotoxicity induced by formaldehyde and acrolein mixtures on human bronchial epithelial BEAS-2B cells. Environment International, 143, 105943. https://doi.org/10.1016/j.envint.2020.105943

## Abbreviations

| | |
|---|---|
| BMDL | benchmark dose lower limit |
| bw | body weight |
| CA | chromosomal aberration |
| CAS | Chemical Abstract Service |
| CEF | Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CHL | Chinese hamster lung |
| CHO | Chinese hamster ovary |
| COE | Council of Europe |
| CONTAM | Panel on Contaminants in the Food Chain |
| DMSO | dimethyl sulfoxide |
| DRF | dose range finding |
| ECHA | European Chemicals Agency |
| ENU | N-ethyl-N-nitrosourea |
| FAF | Panel on Food Additives and Flavourings |
| FAIM | Food Additive Intake Model |
| FC | food category |
| FGE | flavouring group evaluation |

| | |
|---|---|
| FL-no | FLAVIS number |
| GC | gas chromatography |
| GC–FID | gas chromatography–flame ionisation detection |
| GC–MS | gas chromatography–mass spectrometry |
| GLP | good laboratory practices |
| HBGV | health-based guidance values |
| IARC | International Agency for Research on Cancer |
| ICP-MS | inductively coupled plasma-mass spectrometry |
| ip | intraperitoneal |
| IQ | intelligence quotient |
| ISS | Istituto Superiore di Sanità |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LOQ | limit of quantification |
| ML | maximum level |
| MN | micronucleus |
| MNBN | bi-nucleated cells with micronuclei |
| MNPCE | micronucleated polychromatic erythrocytes |
| MOE | margin of exposure |
| MTD | maximum tolerated dose |
| NCE | normochromatic erythrocytes |
| OECD | Organisation for Economic Co-operation and Development |
| P95 | 95th percentile |
| PAHs | polycyclic aromatic hydrocarbons |
| PCE | polychromatic erythrocytes |
| pfu | plaque forming units |
| QSAR | quantitative structure–activity relationship |
| ROS | reactive oxygen species |
| RSD | relative standard deviation |
| SD | standard deviation |
| SF | smoke flavouring |
| TG | test guideline |
| TGR | transgenic rodent |
| TK | thymidine kinase |
| TR | technical requirements |
| TTC | threshold of toxicological concern |
| TWI | tolerable weekly intake |
| UDS | unscheduled DNA synthesis |
| VOC | volatile organic compound |
| WOE | weight of evidence |
| wt | weight |

# Appendix A – Full list of identified and quantified constituents of smoke flavouring Primary Product SF-002

**Table A.1:** Compilation of the 47 identified and quantified volatile constituents in the Primary Product (Documentation provided to EFSA No. 1)

| CAS no | FL-no | Chemical name[a] | Average concentration[b] (wt%) |
|---|---|---|---|
| 64-19-7 | 08.002 | acetic acid | 7.3 |
| 141-46-8 | | acetaldehyde, hydroxy- | 2.4 |
| 498-07-7 | | β-D-glucopyranose, 1,6-anhydro- | 2.3 |
| 64-18-6 | 08.001 | formic acid | 1.8 |
| 116-09-6 | 07.169 | 1-hydroxypropan-2-one (2-propanone, 1-hydroxy-) | 1.6 |
| 5077-67-8 | 07.090 | 1-hydroxybutan-2-one (1-hydroxy-2-butanone) | 1.0 |
| 67-56-1 | | methanol | 0.7 |
| 79-20-9 | 09.023 | methyl acetate (acetic acid, methyl ester) | 0.6 |
| 75-07-0 | 05.001 | acetaldehyde | 0.4 |
| 497-23-4 | former 10.066[c] | furan-2(5*H*)-one (2(5*H*)-furanone) | 0.3 |
| 120-80-9 | 04.029 | benzene-1,2-diol (catechol) | 0.3 |
| 80-71-7 | 07.056 | 3-methylcyclopentan-1,2-dione (2-cyclopenten-1-one, 2-hydroxy-3-methyl-) | 0.3 |
| 91-10-1 | 04.036 | 2,6-dimethoxyphenol (phenol, 2,6-dimethoxy-) | 0.2 |
| 98-01-1 | 13.018 | furfural | 0.2 |
| 50-00-0 | | formaldehyde | 0.2 |
| 67-64-1 | 07.050 | acetone | 0.1 |
| 4451-30-3 | | 1,4:3,6-dianhydro-α-D-glucopyranose | 0.1 |
| 431-03-8 | 07.052 | diacetyl (2,3-butanedione) | 0.08 |
| 90-05-1 | 04.005 | 2-methoxyphenol (phenol, 2-methoxy-) | 0.07 |
| 108-95-2 | 04.041 | phenol | 0.06 |
| 10493-98-8 | | 2-hydroxycyclopent-2-en-1-one | 0.1 |
| 67-47-0 | 13.139 | 5-hydroxymethylfurfuraldehyde (5-hydroxymethylfurfural) | 0.1 |
| 123-38-6 | 05.002 | propanal | 0.04 |
| 78-93-3 | 07.053 | butan-2-one (2-butanone) | 0.04 |
| 107-31-3 | 09.642 | methyl formate | 0.04 |
| 118-71-8 | 07.014 | maltol | 0.03 |
| 107-18-6 | | 2-propen-1-ol | 0.03 |
| 620-02-0 | 13.001 | 5-methylfurfural (2-furancarboxaldehyde, 5-methyl-) | 0.03 |
| 106-44-5 | 04.028 | 4-methylphenol (*p*-cresol) | 0.03 |
| 95-48-7 | 04.027 | 2-methylphenol (phenol, 2-methyl-) | 0.02 |
| 554-12-1 | 09.134 | methyl propionate | 0.02 |
| 109-87-5 | 06.074 | dimethoxymethane (methylal) | 0.01 |

| CAS no | FL-no | Chemical name[a] | Average concentration[b] (wt%) |
|---|---|---|---|
| 600-14-6 | 07.060 | pentan-2,3-dione (2,3-pentanedione) | 0.01 |
| 120-92-3 | 07.149 | cyclopentanone | 0.01 |
| 97-99-4 | 13.020 | tetrahydrofurfuryl alcohol (tetrahydro-2-furanmethanol) | 0.01 |
| 64-17-5 | 02.078 | ethanol | 0.01 |
| 3102-33-8 | 07.044[d] | pent-3-en-2-one (3-penten-2-one, (*E*)-) | 0.01 |
| 2311-46-8 | 09.062 | isopropyl hexanoate | 0.005 |
| 634-36-6 | 04.084 | 1,2,3-trimethoxybenzene | 0.005 |
| 623-42-7 | 09.038 | methyl butyrate (butanoic acid, methyl ester) | 0.004 |
| 123-72-8 | 05.003 | butanal | 0.003 |
| 576-26-1 | 04.042 | 2,6-dimethylphenol (phenol, 2,6-dimethyl-) | 0.003 |
| 1489-69-6 | | cyclopropanecarboxaldehyde | 0.003 |
| 814-78-8 | | 3-buten-2-one, 3-methyl- | 0.002 |
| 71-23-8 | 02.002 | propan-1-ol (1-propanol) | 0.002 |
| 590-86-3 | 05.006 | 3-methylbutanal (butanal, 3-methyl-) | $2.7 \times 10^{-4}$ |
| 109-49-9 | 07.162 | hex-5-en-2-one (5-hexen-2-one) | $1.67 \times 10^{-4}$ |

(a): In case a constituent of the Primary Product is an authorised flavouring substance (FL-no), the assigned chemical name corresponds to the respective entry in the EU Union List of flavourings. Deviating chemical names reported by the applicant in the dossier are given in brackets, if applicable.
(b): The values reported are claimed to be obtained from a duplicate analysis, however, the individual values nor the batch IDs were provided.
(c): 'Former FL-number' refers to substances that were initially included in the evaluation programme but were not included or were removed/withdrawn from the Union List.
(d): [FL-no: 07.004] refers to the mixture of *E/Z* stereoisomers of pent-3-en-2-one.

# Appendix B – Genotoxicity data available on 6 individual components for which a (potential) concern for genotoxicity is identified

The data on the six substances discussed in this Appendix relate to:

i) furan-2(5*H*)-one (CAS No. 497-23-4) and benzene-1,2-diol (catechol, CAS No. 120-80-9) for which a concern for genotoxicity has been identified;
ii) four substances described in Section 3.4.1 for which a potential concern for genotoxicity has been identified, i.e. hydroxyacetaldehyde (CAS No. 141-46-8), acetaldehyde (CAS No. 75-07-0), formaldehyde (CAS No. 50-00-0) and 2-propenal (acrolein CAS No 107-02-8).

## B.1. Furan-2(5*H*)-one (CAS No. 497-23-4, former [FL-no:10.066])

Furan-2(5*H*)-one (former [FL-no:10.066]) was evaluated as genotoxic *in vivo* (EFSA FAF Panel, 2019).

The applicant estimated the exposure to furan-2(5*H*)-one from the use of the Primary Product, which is above the TTC value of 0.0025 μg/kg body weight (bw) per day and therefore presents a safety concern for genotoxicity.

The applicant further assessed the potential risk to furan-2(5*H*)-one, comparing the exposure to this substance (from the use of the Primary Product) with the dose of the Primary Product, which did not induce genotoxic effects in *in vivo* studies.

The applicant derived a NOAEL of 125 mg/kg bw per day (the highest dose at which no statistically significant increase in tail intensity was observed) from the *in vivo* comet assay, which was evaluated by the FAF Panel in flavouring group evaluation (FGE) 217Rev2 (EFSA FAF Panel, 2019). A dose–response modelling was applied to the same study and a BMDL of 80.1 mg/kg bw was calculated. Based on this BMDL value, the applicant calculated a MOE of approximately 19000. The applicant speculated that the MOE for carcinogenicity might be even higher if there were tumour data.

**Conclusion**: the Panel considered that furan-2(5*H*)-one displayed a genotoxic activity *in vivo* based on a comet assay in liver (EFSA FAF Panel, 2019) and in a MN assay in liver which was evaluated in the context of other smoke flavourings (EFSA FAF Panel, 2023a,b,c). From these EFSA opinions, no evidence is available to prove that furan-2(5*H*)-one induces chromosomal damage via a threshold-based mechanism. In addition, the Panel considered that the derivation of reference points from the available genotoxicity studies and the calculation of a MOE is not appropriate (as described in Section 3.4.1). Since the exposure to furan-2(5*H*)-one exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 16), the Panel considered that a safety concern emerges for this component.

## B.2. Benzene-1,2-diol (catechol) [FL-no: 04.029] (CAS No. 120-80-9)

The applicant calculated a MOE based on the results of the *in vivo* genotoxicity studies on the whole mixture (Labcorp, 2022a,b). As described in Section 3.4.1, the Panel considered this approach not appropriate.

The Panel noted that benzene-1,2-diol was evaluated as flavouring substance by the Council of Europe (CoE) before 2000. Therefore, no assessment of this substance was performed by EFSA (according to Regulation (EC) No 1565/2000[20]). In the evaluation by CoE,[21] no details are given to acknowledge whether genotoxicity has been assessed.

Information on genotoxicity were reported by IARC (1999), OECD (2003) and Health Council of the Netherlands (2011). Experimental genotoxicity data[22] have been evaluated more recently by ECHA (ECHA, 2016a), leading to a standardised classification for genotoxicity as 'Muta 2' for this substance.[23]

**Conclusion**: Based on experimental *in vitro* and *in vivo* data on benzene-1,2-diol (including studies where animals were exposed via oral route) a concern for genotoxicity *in vivo* is identified. A safety concern emerges since the exposure to benzene-1,2-diol exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 16).

[20] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council (Text with EEA relevance). OJ L 180, 19.7.2000, p. 8–16
[21] https://book.coe.int/en/health-protection-of-the-consumer/2208-chemically-defined-flavouring-substances.html
[22] https://echa.europa.eu/documents/10162/26132aa6-5033-9928-92b1-cdce3f8c2652 (background document to the opinion proposing harmonised classification and labelling at EU level of 1,2-dihydroxybenzene).
[23] https://echa.europa.eu/da/information-on-chemicals/cl-inventory-database/-/discli/details/67398

## B.3. Hydroxyacetaldehyde (CAS No. 141-46-8)

The applicant provided *in vitro* analysis, but no experimental data on this substance.

The Panel identified in the literature the following papers reporting studies on hydroxyacetaldehyde.

In Hengstler et al. (1994) human peripheral mononuclear blood cells were exposed to hydroxyacetaldehyde, for 2 h at concentrations between 1 and 10 mM. A concentration-dependent increase in DNA crosslinks was observed using the alkaline filter elution (modified protocol to specifically detect DNA crosslinks); the study also showed that the crosslinks were mainly DNA-protein; DNA single-strand breaks were also produced. The Panel considered this study as reliable with restrictions because the test is not sufficiently standardised and the results of limited relevance.

In Denkel et al. (1986) negative results applying alkaline elution were obtained exposing CO631 (SV40-transformed Chinese Hamster) cells to concentrations up to a cytotoxicity of 30%. Alkaline elution was also applied to detect DNA damage in liver from rats exposed to a single oral dose of hydroxyacetaldehyde. Also, this *in vivo* study did not show an effect of the compound. Of note, the method applied is not suitable to detect DNA crosslinks. Considering that the compound is suspected to be a crosslinking agent, these negative results in *in vitro* and *in vivo* studies are of low relevance.

In the same article, the bacterial reverse mutation assay was applied to test the compound up to the concentration of 40 μmol/plate in *S. Typhimurium* TA100, TA 98 and TA1535. The authors considered the assay weakly positive in the strain TA100 without metabolic activation, although the highest increase of revertants was only approximately 1.5 times. The Panel considered this part of the study as reliable with restrictions (because the compound was tested only on three strains) and the results as equivocal.

Garst et al. (1983) tested the substance in a bacterial reverse mutation assay on *S. Typhimurium* TA 100 with and without S9 fraction reporting positive results. The insufficient information regarding the methods and the results, which are only described as positive or negative, does not allow the evaluation of the reliability of this study.

**Conclusion:** Given the reactivity of the substance towards DNA and the equivocal results of a bacterial gene mutation assay, *in vitro* studies addressing gene mutations as well as structural and numerical chromosomal aberrations would be needed to evaluate the genotoxic potential of the substance, since the exposure to hydroxyacetaldehyde exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 16).

## B.4. Acetaldehyde [FL-no: 05.001] (CAS No. 75-07-0)

The applicant calculated a MOE based on the results of the *in vivo* genotoxicity studies on the whole mixture (Labcorp, 2022a,b). As described in Section 3.4.1, the Panel considered this approach not appropriate.

The Panel noted that acetaldehyde (JECFA No. 80) was evaluated as flavouring substance by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) before 2000 (JECFA, 1999). Therefore, no assessment of this substance was performed by EFSA (according to Regulation (EC) No 1565/2000). In the evaluation by JECFA, genotoxicity data were not assessed.

Information on genotoxicity was reported by IARC (1999, 2012a). Experimental genotoxicity data have been evaluated more recently by ECHA (ECHA, 2016b), leading to a standardised classification for genotoxicity as 'Muta 2' for this substance. There is supportive evidence for genotoxic potential *in vivo* based particularly on positive micronuclei formation in both rats and mice, albeit following intraperitoneal (ip) administration. The Panel noted that none of the *in vivo* genotoxicity studies evaluated by ECHA were performed via oral administration. Since in the *in vivo* studies assessed, animals were administered via ip injection, it is possible that this route overwhelms detoxification mechanisms and may not reflect responses to oral administration. Nevertheless, there is, at least, the potential for direct genotoxicity *in vivo* at the point of contact.

**Conclusion**: based on the experimental data acetaldehyde is genotoxic *in vitro* and *in vivo* following ip administration. These findings would require *in vivo* genotoxicity studies following oral administration. These studies should address gene mutations and structural and numerical chromosomal aberrations in particular at the site of contact, since the exposure to acetaldehyde exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 16).

## B.5. Formaldehyde (CAS No. 50-00-0)

The applicant calculated a MOE based on the results of the *in vivo* genotoxicity studies on the whole mixture (Labcorp, 2022a,b). As described in Section 3.4.1, the Panel considered this approach not appropriate.

Formaldehyde was evaluated by the EFSA AFC Panel (2007) as a preservative during the manufacture and preparation of food additives. For the assessment of genotoxicity, the AFC Panel referred in particular to WHO (2002) and BfR (2006). The genotoxicity of formaldehyde has been extensively reviewed by IARC (2006, 2012b). Formaldehyde has been evaluated also by EFSA FEEDAP Panel (2014a,2014b) and by ECHA (2012). ECHA (2012) classified formaldehyde as 'Muta 2' and considered that '*The route(s) of exposure should not be stated in the hazard statement as it is not proven that other routes than inhalation can be excluded*". Overall, formaldehyde was shown to induce DNA lesions (DNA strand breaks, adducts, DNA-protein crosslinks) and mutagenic effects (gene mutations, structural chromosomal aberrations and micronuclei) in a large number of *in vitro* studies. *In vivo*, after inhalation exposure, genotoxic effects were reported at the first site of contact (nasal tissues) of experimental animals. Epidemiological studies in occupationally exposed populations indicated the induction of genotoxic effects in the tissues directly exposed after inhalation. The issue of possible systemic genotoxicity remains controversial: conflicting results were reported in a wide range of studies conducted on experimental animals and on exposed human populations. The available data set is essentially based on inhalation studies. In a non-guideline study (Migliore et al., 1989) a single oral administration of 200 mg/kg bw induced significant increases of micronuclei frequency in forestomach, duodenum, ileum and colon. While formaldehyde is a recognised genotoxic carcinogen by inhalation, a conclusive assessment of genotoxicity and carcinogenicity after oral exposure is not possible, based on the available data.

**Conclusion:** based on the experimental data formaldehyde is genotoxic *in vitro* and *in vivo* after inhalation exposure. These findings would require *in vivo* genotoxicity studies following oral administration. These studies should address gene mutations and structural and numerical chromosomal aberrations in particular at the site of contact, since the exposure to formaldehyde exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 16).

## B.6. 2-Propenal (acrolein) (CAS No. 107-02-8)

Acrolein has been recently assessed by IARC (IARC, 2021). Based on the data reported by IARC, acrolein is a highly electrophilic agent which readily reacts with a variety nucleophilic cell component. On the cellular level, acrolein exposure has diverse toxic effects, including DNA and protein adducts, oxidative stress, mitochondrial disruption, membrane damage, endoplasmic reticulum stress. Acrolein was reported to induce DNA adducts and DNA–protein crosslinks in different cell lines and human primary cell cultures. It inhibits proteins involved in the three major DNA-repair pathways. In addition, acrolein induces depletion of glutathione and increased generation of ROS (reactive oxygen species) indicative of an oxidative stress.

Acrolein has been tested in several studies using different *Salmonella* strains with mixed results. In strains, used to detect the base-pair substitutions, negative results were reported with TA1535 (eight studies available) applying the standard protocol and the preincubation method and positive results with TA100 in 4 out of 13 studies available, only in the absence of metabolic activation. In strains used to detect frameshift mutations negative results were reported in three studies with TA1537, in two studies with TA1538 and in a study with TA97, while positive results were obtained in 3 out of 10 studies with TA98. Only one study is available with negative results in TA102, a strain that is used specifically for the detection of crosslinking agents (IARC, 2021).

A concentration-related increase in 6-thioguanine resistant mutants was reported in fibroblast cultures derived from xeroderma pigmentosum (XP) patients, but not in normal human fibroblast cultures (Curren et al., 1988). Positive results for *Hprt* mutations were also reported in two studies in Chinese hamster lung fibroblasts (V79) (Smith et al., 1990; Gardner et al., 2004) and in Chinese hamster ovary cells (CHO) (Cai et al., 1999), negative results for *Hprt* mutations in CHO cells were reported in another study (Foiles et al., 1990). A significant increase in the frequency of *Tk* mutations was reported in mouse lymphoma (L5178Y) cells (Demir et al., 2011), but a negative response was reported for the induction of *cII* mutations in mouse embryonic fibroblasts from the Big Blue mouse (Kim et al., 2007).

Negative results for chromosomal aberrations were described in CHO cells in presence and absence of metabolic activation (Galloway et al., 1987). Positive results with the MN test were reported in lung A549 cells and BEAS-2B cells in two studies (Zhang et al., 2018; Zhang et al., 2020).

The induction of DNA single-strand breaks by acrolein, evaluated by alkaline elution and by comet assay, were reported in different cell lines in a number of studies reported by IARC (2021). The results were considered by the Panel as of low or limited relevance.

Few *in vivo* studies in rodents were reported by IARC (2021). In an *in vivo* MN assay, acrolein induced a weak, but statistically significant increase in micronucleated polychromatic erythrocytes in the bone marrow of male Sprague–Dawley rats treated at a single dose of 5 mg/kg bw per day by gavage, six times per week, for 30 days (Aydın et al., 2018). The Panel considered this study to be reliable with restrictions and the results of limited relevance.

No significant increase in the frequency of dominant lethal mutations was observed in male ICR/Ha Swiss mice exposed to acrolein after a single ip injection (Epstein et al., 1972).

In an *in vivo* MN study reported by NTP (NTP, 2006), no increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood of male or female B6C3F1 mice treated with acrolein at different doses up to 10 mg/kg bw per day by gavage for 14 weeks. No significant clinical signs were reported in the tested animals, although 10 mg/kg bw was selected as the maximum tolerated dose in a range finding study where a reduced survival was observed at 20 mg/kg. The Panel considered the study results as inconclusive because there was no demonstration of bone marrow exposure.

**Conclusions:** based on the experimental data acrolein is genotoxic *in vitro*. The Panel considered that the available *in vivo* studies are inconclusive or of limited relevance and do not allow assessment of e genotoxicity of acrolein *in vivo*. Therefore, *in vivo* genotoxicity studies following oral administration would be required. These studies should address gene mutations and structural and numerical chromosomal aberrations in particular at the site of contact.

# Appendix C – Approach for assessing reliability and relevance of genotoxicity studies

Evaluation of data quality for hazard/risk assessment includes evaluation of reliability of studies and relevance of study results (Klimisch et al., 1997; ECHA, 2011; EFSA Scientific Committee, 2011, 2017b, 2021b). Reliability is assessed using a scoring system based on published criteria (Klimisch et al., 1997) described in the following Section. In a second step, the relevance (high, limited or low) of study results is assessed based on several aspects (genetic endpoint, route of administration, status of validation of the assay, etc.) discussed in Section C.2, and also taking into account the assessment of the reliability of the study.

Only studies with acceptable relevance (high or limited) are considered in the weight of evidence approach (WoE). Genotoxicity studies evaluated as of low relevance are not further considered in the WoE.

## C.1. Evaluation of reliability of results of genotoxicity studies – general considerations

The scoring system for reliability is based on the scoring system of Klimisch et al. (1997). Reliability is defined by Klimisch as 'evaluating the inherent quality of a test report or publication relating to preferably standardised methodology and the way that the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings'. In assigning the reliability score, the compliance with the OECD Test Guidelines (TGs) or standardised methodology and the completeness of the reporting should be considered.

The reliability scores are:

1) reliable without restriction,
2) reliable with restrictions,
3) reliability insufficient,
4) reliability cannot be evaluated.

*1. Reliable without Restriction* 'This includes studies or data from the literature or reports which were carried out or generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline (preferably performed according to GLP) or in which all parameters described are closely related/comparable to a guideline method'.

*2. Reliable with Restrictions* 'This includes studies or data from the literature, reports (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable'.

*3. Reliability Insufficient*[24] 'This includes studies or data from the literature/reports in which there are interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (...) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert judgment'.

*4. Reliability cannot be evaluated* [25] 'This includes studies or data from the literature, which do not give sufficient experimental details, and which are only listed in short abstracts or secondary literature (books, reviews, etc.)'.

## C.2. Evaluation of relevance of results of individual genotoxicity studies - general considerations

The relevance of the test system and test results are reported separately.

The relevance of the test systems (high, limited, low) is principally based on the following criteria:

[24] Klimisch et al. (1997) used the term 'Not reliable' however, 'Reliability Insufficient' was considered more appropriate.
[25] Klimisch et al. (1997) used the term 'Not assignable' however, 'Reliability cannot be evaluated' was considered more appropriate.

- Genetic endpoint: higher relevance is given to studies providing information on apical endpoints, i.e. gene mutations, structural and numerical chromosomal alterations. Supporting information may be obtained from indicator assays; exception is the *in vivo* Comet assay that is considered with high relevance when applied as follow-up to a positive *in vitro* result (as recommended by the EFSA Scientific Committee (2011)).
- Status of validation of the test system (e.g. (in order of decreasing relevance) availability of an OECD TG consolidated or in the course of development or internationally recommended protocol, validation at national level only).

The relevance of the study results (high, limited, low) are principally based on the following criteria:

- Reliability of studies: the results of studies with reliability that are insufficient or which cannot be evaluated (see points 3–4 in Section C.1) are considered of low relevance.
- Relevance of the test system.
- Route of administration: higher relevance is given to oral versus intravenous or subcutaneous injection and inhalation exposure in case of *in vivo* studies. Lower relevance is given to studies using the intraperitoneal route, which is not physiological and not recommended by OECD TGs.
- Biological relevance of the test results, considering: purity of the test substance; the metabolic capabilities of the test system; the bioavailability of the test substance, with particular consideration of the evidence of target tissue exposure in tests *in vivo*: negative results without evidence of target tissue exposure are considered as inconclusive and their relevance low; the interference of high cytotoxicity; the reproducibility of test results.

## Appendix D – Genotoxicity studies on the Primary Product (whole mixture) evaluated by the CEF Panel (EFSA CEF Panel, 2009)

**Table D.1:** Summary of *in vitro* genotoxicity studies on Zesti Smoke Code 10 (SF-002) including re-evaluation of reliability and relevance by the FAF Panel (approach described in Appendix C)

| Name | Test System *in vitro* | Test Object | Concentrations and Test Conditions | Result | Reliability/Comments | Relevance of test system/ Relevance of the result | Reference |
|---|---|---|---|---|---|---|---|
| Zesti Smoke Code 10 | Bacterial Reverse Mutation test | *S.* Typhimurium TA98, TA100, TA1535, TA1537, TA102 | Experiment 1: 1.6–5,000 μg/plate (+/-S9, plate incorporation)<br>Experiment 2: 78.1–5,000 μg/plate (+/-S9, plate incorporation) | Positive | Reliable without restrictions. Study performed according to OECD TG 471 and in compliance with GLP | High/High | Covance (2004) |
| | *In vitro* mammalian cell gene mutation test in mouse lymphoma cells | L5178Y TK $^{+/-}$ mouse lymphoma cells | 100–225 μg/mL (3 h, -S9)<br>200–450 μg/mL (3 h, +S9) | Positive | Reliable without restrictions. Study performed according to OECD TG 476 (applicable at that time, now OECD TG 490) and in compliance with GLP | High/High | Covance (2006a) |
| | *In vitro* mammalian chromosomal aberration test | Human peripheral blood lymphocytes | Experiment 1: 219.9, 343.6, 671.1 μg/mL (3 + 17 h, +S9)<br>219.9, 343.6, 429.5 μg/mL (3 + 17 h, -S9)<br>Experiment 2: 327, 532.4, 737 μg/mL (3 + 17 h, +S9)<br>313.2, 368.5, 433.5 μg/mL (20 h, -S9) | Positive | Reliable with restrictions (200 or less metaphases/concentration instead of 300 were scored; historical data for positive control not reported). Study performed according to OECD TG 473 and in compliance with GLP. | High/Limited | Covance (2006b) |

**Table D.2:** Summary of *in vivo* genotoxicity studies on Zesti Smoke Code 10 (SF-002) including re-evaluation of reliability and relevance by the FAF Panel (approach described in Appendix C)

| Name | Test System *in vivo* | Test object route | Doses (mg/kg bw per day) | Result | Reliability/Comments | Relevance of test system/ Relevance of the result | Reference |
|---|---|---|---|---|---|---|---|
| Zesti Smoke Code 10 | Micronucleus assay in bone marrow | Sprague Dawley Crl:CD® (SD) rats; M Oral | 2,000[(a)] | Inconclusive (Negative, but without sufficient demonstration of bone marrow exposure) | Reliable with restrictions (limited number of cells analysed; higher doses might have been applied; lack of demonstration of bone marrow exposure). Study performed according to OECD TG 474 and in compliance with GLP | High/Low | Covance (2006c) |
| | UDS assay in liver | Sprague Dawley Crl:CD®(SD) rats; M Oral | 2,000[(b)] | Negative | Reliable without restrictions. Study performed according to OECD TG 486 and in compliance with GLP | Low/Low | Covance (2005) |

M: males.
(a): Animals dosed once daily on two consecutive days; sampling at 24 h after the last dose administration.
(b): One administration with sampling at: 2–4 h and 12–14 h.

# Appendix E – New genotoxicity studies on the Primary Product (whole mixture)

**Table E.1:** Summary of new *in vitro* genotoxicity studies on Zesti Smoke Code 10 (SF-002)

| Name | Test system *in vitro* | Test object | Concentrations[(a)] of substance and test conditions | Result | Reliability/Comments | Relevance of test system/ Relevance of the result | Reference |
|---|---|---|---|---|---|---|---|
| Zesti Smoke Code 10 | Reverse Mutation test | *S.* Typhimurium TA98, TA100, TA 102, TA1535 and TA1537 | Experiment 1: 5–5,000 μg/plate (+/– S9, plate incorporation)<br>Experiment 2: 50–5,000 μg/plate (+/– S9, preincubation) | Positive | Reliable without restrictions. Study performed according to OECD TG 471 and in compliance with GLP. | High/High | Labcorp (2021a) |
| | Micronucleus assay | Human peripheral blood lymphocytes | 120, 225 and 325 μg/mL (3 + 21 h, -S9)<br>140, 270, 350 and 400 μg/mL (3 + 21 h, +S9)<br>120, 180, 200 and 220 μg/mL (24 h + 24 h, -S9) | Positive | Reliable without restrictions. Study performed according to OECD TG 487 and in compliance with GLP. | High/High | Labcorp (2021b) |

(a): For the *in vitro* MN assay, the given concentrations are those for the cultures that were scored for micronuclei.

**Table E.2:** Summary of new *in vivo* genotoxicity studies on Zesti Smoke Code 10 (SF-002)

| Name | Test system *in vivo* | Test object route | Doses (mg/kg bw per day) | Result | Reliability/Comments | Relevance of test system/ Relevance of the result | Reference |
|---|---|---|---|---|---|---|---|
| Zesti Smoke Code 10 | Micronucleus assay in bone marrow | Han Wistar rats; M<br><br>gavage | 875, 1,750 and 3,500[(a)] | Inconclusive (Negative, but without demonstration of sufficient bone marrow exposure) | Reliable with restrictions (no demonstration of sufficient bone marrow exposure). Study performed in compliance with GLP and according to OECD TG 474. | High/Low | Labcorp (2022a) |
| | Gene mutation assay in liver and glandular stomach | Muta™Mouse (*lacZ/GalE*) $CD_2$-LacZ80/ HazfBR SPF transgenic mice; M & F<br><br>diet | 1,024, 2,011 and 4,206 for M; 2,491, 4,894 and 1,0284 for F[(b)] | Negative | Reliable without restrictions. Study performed in compliance with GLP and according to OECD TG 488 | High/High | Labcorp (2022b) |

bw: body weight; M: males; F: females.
(a): The Primary Product was administered once daily on 2 consecutive days; sampling at 24 h after the last administration.
(b): Doses calculated from feed concentrations of 6,250, 12,500 and 25,000 mg/kg diet for male animals and 12,500, 25,000 and 50,000 mg/kg diet for female animals.

## Annex A – Exposure assessment results

- Annex A1 Occurrence data per food category considered in FAIM, (mg/kg).
- Annex A2 Total estimated exposure of Zesti Smoke Code 10 (SF-002) for the proposed maximum use level exposure assessment scenario using FAIM, per population group and survey: mean and 95th percentile (mg/kg bw per day).
- Annex A3 Total estimated exposure of Zesti Smoke Code 10 (SF-002) for the expected typical use level exposure assessment scenario using FAIM, per population group and survey: mean and 95th percentile (mg/kg bw per day).
- Annex A4 Proposed food categories and use levels linked to FoodEx2 foods, considered within DietEx and their dilution factors (mg/kg or mg/L).
- Annex A5 Total estimated exposure of Zesti Smoke Code 10 (SF-002) for the proposed maximum use level exposure assessment scenario using DietEx, per population group and survey: mean and 95th percentile (mg/kg bw per day).
- Annex A6 Total estimated exposure of Zesti Smoke Code 10 (SF-002) for the expected typical use level exposure assessment scenario using DietEx, per population group and survey: mean and 95th percentile (mg/kg bw per day).
- Annex A7 Main food categories contributing to exposure to Zesti Smoke Code 10 (SF-002) at the proposed maximum use level exposure assessment scenario using DietEx (> 5% to the total mean exposure).
- Annex A8 Main food categories contributing to exposure to Zesti Smoke Code 10 (SF-002) at the expected typical use level exposure assessment scenario using DietEx (> 5% to the total mean exposure).
- Annex A9 Qualitative evaluation of the influence of standard uncertainties on the dietary exposure estimates of the Primary Product.

Annex A can be found in the online version of this output, in the 'Supporting information' section.

## Annex B – Genotoxicity assessment of the identified components in the Primary Product

Annex B can be found in the online version of this output, in the 'Supporting information' section.