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Scientific opinion on the renewal of the authorisation of SmokEz C-10 (SF-005) as a smoke flavouring Primary Product

EFSA Panel name on Food Additives and Flavourings (FAF),
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Abstract

The EFSA Panel on Food Additives and Flavourings (FAF) was requested to evaluate the safety of the smoke flavouring Primary Product SmoKEz C-10 (SF-005), 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. SmoKEz C-10 is obtained by pyrolysis of maple, oak, hickory, ash, birch, beech and cherry 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 0.01 to 5.1 mg/kg body weight (bw) per day at the mean and from no dietary exposure to 18.1 mg/kg bw per day at the 95th percentile. The Panel concluded that five 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(*5H*)-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: benzene-1,2-diol, catechol, furan-2(5*H*)-one, genotoxicity, SmoKEz C-10, SF-005, smoke flavouring Primary Product

Requestor: European Commission

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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 there from 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 SmokEz C-10 (SF-005) 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 SmokEz C-10 (SF-005), 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 SmokEz C-10 (SF-005).

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 SmokEz C-10 (SF-005) in 2009 (EFSA CEF Panel, 2009), updated in 2012 (EFSA CEF Panel, 2012).

Following the safety assessment from EFSA, SmokEz C-10 was authorised in the European Union and assigned the unique code 'SF-005', 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 SmokEz C-10 (SF-005) 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 SmokEz C-10 (SF-005).

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 39–39e 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 April to 24 April 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 SmokEz C-10 (SF-005) 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 SmokEz C-10 is hardwood sawdust from hard maple (*Acer saccharum*) (25–60%), white oak (*Quercus alba*) (10–40%), hickory (*Carya ovata*) (10–25%) and low quantities of other wood species: white/black ash (*Fraxinus americana*) (0–15%), birch (*Betula papyrifera* and

[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/questions/EFSA-Q-2022-00416

Betula alleghanisensis) (0–15%), beech (*Fagus grandifolia*) (0–15%) and cherry (*Prunus serotina*) (0–15%) (Documentation provided to EFSA No. 1). 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; the formed smoke vapour is condensed and the condensate is transferred into a storage tank. The addition of water (approximately 40%) results in the formation of three distinct phases. The lower tarry phase (about 15%) and the upper oily phase (generally < 1%) are discarded, the remaining aqueous phase is sent to another storage tank. The Primary Product is obtained after separation from the sedimented tar and subjecting the aqueous phase to a filtration step (1 μm).

The applicant submitted a description of the manufacturing process, including 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 SmokEz C-10.

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

The applicant indicated that the smoke flavouring SmokEz C-10 has not been evaluated by regulatory bodies other than EFSA. Regarding the existing authorisations in non-EU countries, the applicant stated that SmokEz C-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*' (Documentation provided to EFSA No. 1). The Primary Product has a pH ranging from 2.15 to 2.6, a viscosity (at 25°C) ranging from 2 to 3 cP, a refraction index ranging from 23 to 27 °BRIX and a density (at 20°C) of approximately 1,050 g/L. The applicant described the Primary Product as '*soluble 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.	1,053	≤ 28.4	20.0	< 8.4	≥ 0.7	–	≥ 0.7	70.9	29.1	68.7	≥ 70.4%

n.a: not available (i.e. the applicant did not specify the batch number).
(1): Calculated as: 100 wt% – 70.9 wt% (water) – 0.7 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 3 replicates (individual values: 71.4, 70.6, 70.6 wt%; SD (±) 0.5; RSD (%): 0.7).
(5): Average of 3 replicates (individual values: 28.6, 29.4, 29.4 wt%; SD (±) 0.2; RSD (%): 1.6.
(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]: ≥ 50 (wt%).
(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 101,3 kPa, and consequently, they 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'. 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 (48 in total) have been quantified by the applicant using appropriate methods and amounted to 20 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 0.7 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 0.7 wt%. Accordingly, the amount of total volatiles can be estimated to be not higher than 28.4 wt% (calculated as 100 wt% – 70.9 wt% water – 0.7 wt% tar). Consequently, the identified volatiles constitute at least 70.4 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 300 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	SD
Acids (wt%) (as acetic acid)	10.8[(a)]	1.0
Carbonyls (wt%) (as 2-butanone)	15.1[(a)]	4.4
Phenols (wt%) (as 2,6-dimethoxyphenol)	1.11[(a)]	0.12
Water (wt%)	70.9[(b)]	0.5

(a): Values are based on analysis of 300 batches.
(b): Values are based on data from three replicates.

The Panel noted that in the current renewal application, the water content of the Primary Product amounted to 70.9 ± 0.5 wt%, whereas, in the opinion issued by EFSA in 2009, the water content amounted to 67 wt%. The Panel did not consider this inconsistency of relevance for the safety assessment.

Concentrations of arsenic, cadmium, lead and mercury were determined by ICP-MS and were submitted to EFSA. The analyses were performed on 24 batches of the Primary Product (Table 3) (Documentation provided to EFSA No. 1).

The Panel noted that in all investigated batches, the levels of arsenic and mercury were below the limit of quantifications (LOQs), i.e. < 0.1 mg/kg and < 0.005 mg/kg for As and Hg, respectively. Cadmium in one batch was below the LOQ of 0.01 mg/kg, and in the other 23 batches above the LOQ, with the highest measured value being 0.06 mg/kg. Lead in 20 batches was below the LOQ of 0.05 mg/kg, in two batches at the level of the LOQ, and in two batches above the LOQ, with the highest measured value being 0.41 mg/kg.

Table 3: Toxic elements reported for 24 batches of the Primary Product (Documentation provided to EFSA No. 2)

	Batch no. (mg/kg)																								Average (mg/kg)	SD
	1008942496	1111942426	1207942494	0111042432	0226042413	0330042442	0409042418	0508042444	0606042455	0710042470	040002151582	040002201269	040002236373	040002279592	040002318174	040002338064	040002381426	040002450317	040002497588	040002534300	040002600632	040002625703	040002669659	040002707615		
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	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	–
Cadmium (Cd)	0.02	0.04	0.05	0.03	0.03	0.02	0.03	0.04	0.02	0.03	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.03	0.05	< 0.01	0.06	0.04	0.02	0.02	< 0.03	< 0.01
Lead (Pb)	0.05	< 0.05	< 0.05	0.14	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.41	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.07	< 0.07
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	< 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

GC-MS and 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), formic acid (CAS no.: 64-18-6) and glyceraldehyde (CAS no.: 497-09-6) were not detectable through a direct GC–MS analysis, and therefore, they were derivatised with (perfluorophenyl)hydrazine, methanol and phenylboronic acid, 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, 48 constituents were identified and quantified in the Primary Product (Appendix A, Table A.1). The lowest concentration reported by the applicant was 9×10^{-5} wt% (0.9 mg/kg) for 3-methylbutanal (CAS no: 590-86-3). 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. The 20 principal volatile constituents of the Primary Product are presented in Table 4.

The applicant reported 243 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 SmokEz C-10 accounted for 17.5 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 SmokEz C-10 was not adequately quantified, and that the fraction of identified and quantified volatiles accounted for approximately 20 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 SmokEz C-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 applications, 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	10.2	6.7
498-07-7		β-D-glucopyranose, 1,6-anhydro-	2.5	2.0[#]
64-18-6	08.001	Formic acid	1.6	1.4
141-46-8		Acetaldehyde, hydroxy-	1.1	1.8
116-09-6	07.169	1-hydroxypropan-2-one (2-propanone, 1-hydroxy-)	1.0	2.6
79-09-4	08.003	Propionic acid (propanoic acid)	0.5	0.4
120-80-9	04.029	benzene-1,2-diol (catechol)	0.4	0.4
80-71-7	07.056[d]	3-methylcyclopentan-1,2-dione (2-cyclopenten-1-one, 2-hydroxy-3-methyl-)	0.3	0.2
67-56-1		Methanol	0.3	0.8
50-00-0		Formaldehyde	0.3	0.6
497-23-4	former 10.066[e]	2(5*H*)-furanone	0.3	
75-07-0	05.001	Acetaldehyde	0.3	0.2
107-21-1		Ethylene glycol	0.2	0.1
79-20-9	09.023	Methyl acetate (acetic acid, methyl ester)	0.2	0.3
91-10-1	04.036	2,6-dimethoxyphenol (phenol, 2,6-dimethoxy-)	0.2	0.2
98-01-1	13.018	Furfural	0.2	0.2
107-22-2		Glyoxal	0.1	0.1
78-98-8	07.001	2-oxopropanal (methylglyoxal)	0.1	0.1
108-95-2	04.041	Phenol	0.1	0.1
497-09-6		Propanal, 2,3-dihydroxy-, (*S*)-	0.1	

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

(e): '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.

#: Identified as levoglucosan by HPLC in the previous safety evaluations of the Primary Product (EFSA CEF Panel, 2009).

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 0.7 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 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) 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 have 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, 70.9 wt% of water and 20 wt% of volatile constituents can be considered as identified fractions of the Primary Product. Accordingly, the unidentified fraction of the Primary Product amounts to 9.1 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 SmokEz C-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.

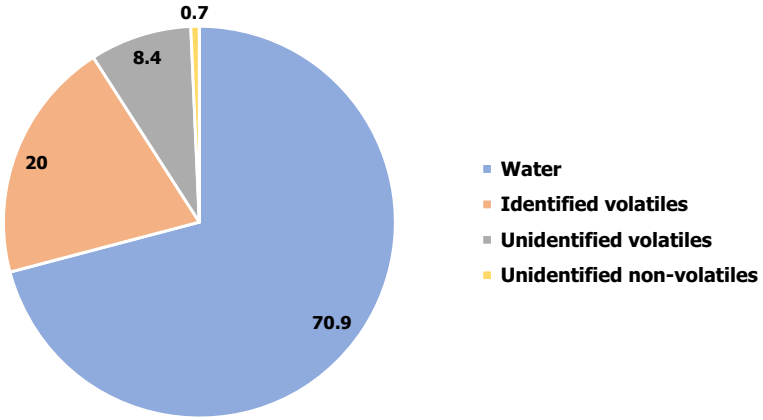

Figure 1: Overall composition of SmokEz C-10 (wt% of Primary Product), as assessed by the Panel. In the pie chart, the symbols '<' and '>' as shown in Table 1 are not taken into account

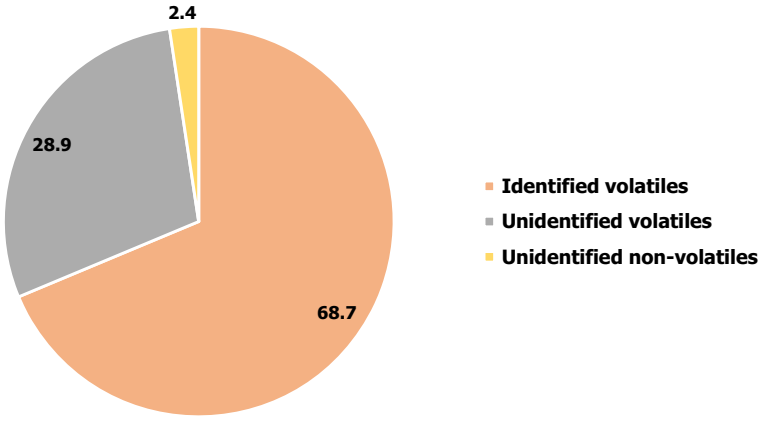

Figure 2: Composition (wt%) of the solvent-free fraction of SmokEz C-10, as assessed by the Panel. In the pie chart, the symbols '<' and '>' as shown in Table 1 are not taken into account

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 3.1.2.4.2), 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 content of 16 PAHs were provided for 24 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 (LOQs) according to Regulation (EC) No 627/2006. All the batches showed PAH levels below LOQ for the method, except for chrysene that was found in one batch (ID: 040002625703) at the LOQ of 0.5 μg/kg.

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, average from 24 batches (for batch numbers, see Table 3) (Documentation provided to EFSA No. 1). Averages and standard deviations were not included as 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)]–0.5
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]pyrene	< 1.0[(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]anthracene	< 0.5[(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)]

(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 the 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 300 batches of the Primary Product 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 content 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 300 batches was acceptable for the parameters monitored. The applicant did not provide information on which specific combinations of wood starting materials are covered by the analysed batches. Nevertheless, the Panel considered that the low standard deviations and the high number of batches analysed indicate that the applicant has adequate control over the production of the Primary Product, resulting in a reproducible product, irrespective of the rather wide ranges of different woods that can be used as starting materials.

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

	Average	SD	RSD (%)
pH	2.22	0.2	9.0
Staining index	55.6	3.2	5.8
Specific gravity	1.057	0.083	7.9
Total acidity (wt%)	10.8	0.95	8.8
Carbonyls (wt%)	15.1	4.5	29.8
Phenols (wt%)	1.11	0.12	10.8
Hydroxyacetaldehyde (wt%)	1.8	0.7	38.9

SD: standard deviation; RSD: relative standard deviation.

In addition, the applicant performed statistical analyses to assess the variability, i.e. means and variances, of 20 volatile constituents in 23 batches of the Primary Product randomly selected from July 2020 to July 2022. 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 20 peaks subjected to statistical analysis covered 68% of the total GC peak area. Based on the reported relative standard deviation, on average < 19% 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 OECD TG 105 (1995) method. Instead, the applicant used published data (or the predictive program 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 9.1 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 nanoparticle 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 SmokEz C-10 is manufactured within its proposed specifications (Documentation provided to EFSA No. 1). 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 SmokEz C-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 materials:			
• Woods	Hard maple (*Acer saccharum*) (25–60%), white oak (*Quercus alba*) (10–40%), hickory (*Carya ovata*) (10–25%), white/black ash (*Fraxinus americana*) (0–15%), birch (*Betula papyrifera* and *Betula alleghanisensis*) (0–15%), beech (*Fagus grandifolia*) (0–15%), and cherry (*Prunus serotina*) (0–15%)	n.a.	Maple (*Acer saccharum*) (25–60%), oak (*Quercus alba*) (10–40%), hickory (*Carya ovata*) (10–25%); ash (*Fraxinus americana*), birch (*Betula papyrifera* and *Betula alleghanisensis*), cherry (*Prunus serotin*a), beech (*Facus grandifolia*): 0–15% (in total)
Identity parameters:			
• Physico-chemical parameters			
– pH	2.15–2.6	2.1–2.6	2.15–2.6
– Density	1.049–1.084 (g/mL)	1.07 kg/L	n.a.
– Refraction index	23–27 (as °BRIX)	n.a.	n.a.
– Staining index	n.a.	n.a.	n.a.
Chemical composition:			
• Chemical classes:			
– Acids	10.5–12.0 wt%	10.5–12.0 wt% (as acetic acid)	10.5–12.0 wt% (as acetic acid)
– Carbonyls	12.0–17.0 wt%	12.0–17.0 wt%	12.0–17.0 wt%
– Phenols	1.0–1.5 wt%	10.0–15.0 mg/mL[(2)]	10.0–15.0 mg/mL
– Water	70.6–71.4 wt%[(3)]	n.a.	60.7–65.1
20 principal constituents of the volatile fraction	See Table 4	n.a.	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			
– lead	0.2 mg/kg	n.a.	< 5.0 mg/kg
– arsenic	< 0.1 mg/kg	n.a.	< 3.0 mg/kg
– cadmium	0.03 mg/kg	n.a.	< 1.0 mg/kg
– mercury	< 0.005 mg/kg	n.a.	< 1.0 mg/kg

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).
(2): Named as 'Smoke flavour compounds.'
(3): The Panel suggests including the water content in the specifications.

The Panel noted that the analytical data for the batches analysed indicated 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 and lead, the applicant proposed specifications limits that are lower than their highest measured values of 0.06 and 0.41 mg/kg for Cd and Pb, respectively (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 specifications 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.

The Panel noted that the ranges of the wood species given in the proposed specifications could be narrowed and requested the applicant to provide more restricted ranges. The applicant replied that these ranges could not be restricted as to encompass all the quantitative variations that the supply chain of the raw material would encounter during the production phase (Documentation provided to EFSA No. 2). The Panel further noted that in the current application wood from cherry (*Prunus serotina*) is listed in the source of raw materials, this also occurs in the former safety assessment (EFSA CEF Panel, 2009) and in the Union List (Regulation (EU) No 1321/2013). Nevertheless, *Prunus serotina* is commonly called 'black cherry', and for this reason, the entry in the proposed specifications should be amended accordingly.

The Panel noted that the applicant did not provide a numerical value for the entry water in the proposed specifications. The range (70.6–71.4%) provided in Table 7 was inserted by the Panel based on the information present in the technical dossier (Documentation provided to EFSA No. 1 and 2). The Panel further noted that for water the numerical values in the current application differ from the values present in the Union List (Regulation (EU) No 1321/2013). The values from the Union List also deviate from the values reported in the previous assessment performed on the Primary Product (EFSA CEF Panel, 2009).

In an additional data request, EFSA had requested the applicant to specify the parameter of staining index of the Primary Product; however, this was not provided (Documentation provided to EFSA No. 2). The panel agreed that the staining index is not fundamental for the safety assessment of 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-year product specifications. The batches are assessed for the parameters of total acids, carbonyls, phenols, °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 were not provided. Based on the average values, all parameters studied remained within their specification ranges over the 2-year storage period, although some batches fell outside of the specifications range for total acids, total carbonyls, total phenols and pH. 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-year range	Average	% change after 2 years of storage	SD	RSD (%)
Total acids (wt%)	10.5–12.0	8.0–12.0	10.4	1.6%	0.2	1.9
Phenols (wt%)	1.0–1.5	0.65–1.5	1.11	−32.0%	0.096	8.6
Carbonyls (wt%)	12.0–17.0	11.0–20	15.1	−22.0%	1.5	9.6
°BRIX	23–27	23–27	25.0	−7.7%	0.3	1.0
pH	2.15–2.6	2.0–3.2	2.22	−7.3%	0.07	3.2

	Spec. at time of manufacture	2-year range	Average	% change after 2 years of storage	SD	RSD (%)
Hydroxyacetaldehyde (wt%)	1.2–3.0	1.2–3.0	1.65	−4.0%	0.2	12.1
Specific gravity	1.049–1.084	1.049–1.084	1.06	−1.3%	0.00	0.0

3.2. Proposed use and use levels

The applicant applied for a renewal of authorisation of the Primary Product SmokEz C-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)]
1.7.5	Processed cheese	Only in 'Processed cheese, sliceable' and 'other processed cheese'	2,000	1,000
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)'	5,000	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'	1,500	600

[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, pp. 16–33.

Food category number	Food category Name	Restrictions/exceptions	Proposed maximum use levels (mg/kg)[a]	Expected typical use levels (mg/kg)[a]
9.3	Fish roe	Only in 'Fish roe'(b)	500	300
12.2	Herbs, spices, seasonings	Only in 'Seasonings and extracts' and 'Coriander seed' and 'Black pepper' and 'Paprika powder'	1,000	500
12.4	Mustard	Only in 'Mustard and related sauces'(b)	100	30
12.5	Soups and broths	Only in 'Potato soup' and 'Legume (beans) soup' and 'Mushroom soup' and 'Cereal products and grains-based soup' and 'Grains soup, dry' and 'Meat and vegetable soup, dry' and 'Mixed vegetables soup, dry' and 'Potato soup, dry' and 'Mixed vegetables soup' and 'Meat soup, with pieces' and 'Meat and vegetable soup, with puree or pieces' and 'Meat and vegetable soup, clear'	100	30
12.6	Sauces	Only in 'Tomato ketchup and related sauces' and 'Barbecue or steak sauces'	9,000	2,000
12.9	Protein products, excluding products covered in category 1.8	Only in 'Meat imitates'	2,000	600
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'	1,000	400
15.2	Processed nuts	Only in 'Almonds' and 'Peanuts'	1,000	400

(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 subcategories, 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 FAIM (Food Additive Intake Model, 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

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, 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, 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, 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, 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, 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 (as registered in the consumption survey) to derive an individual total exposure per day expressed per kilogram body weight. 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).

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.

[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.

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 bw per day)

	Infants (12 weeks to 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	0.1–8.3	2.8–19.9	3.7–19.5	3.7–12.2	2.2–10.2	0.9–6.8
95th percentile	0.4–41.1	11.4–44.8	11.1–45.0	9.7–32.7	5.6–29.3	3.6–19.3
Expected typical use level exposure assessment scenario						
Mean	0.04–2.1	0.8–4.8	1.2–4.8	0.9–3.0	0.5–2.4	0.3–1.6
95th percentile	0.1–8.9	3.0–10.1	3.3–10.3	2.2–7.5	1.3–6.9	1.0–4.1

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 0.1 mg/kg bw per day in infants to 19.9 mg/kg bw per day in toddlers. The 95th percentile of exposure to the Primary product ranged from 0.4 mg/kg bw per day in infants to 45.0 mg/kg bw per day in children.

At the expected typical use levels, the mean exposure ranged from 0.04 mg/kg bw per day in infants to 4.8 mg/kg bw per day in toddlers and children, and the 95th percentile of exposure from 0.1 mg/kg bw per day in infants to 10.3 mg/kg bw per day in children.

The Primary Product is requested for renewal of authorisation in 12 food categories (Table 9). For all these 12 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).

3.2.2.2. 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

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

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 '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 the 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 level of foods 'as consumed' or 'in 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 bw per day)

	Infants (0 weeks to 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	0.01–2.0	0.5–5.1	0.9–4.7	0.7–3.5	0.4–1.8	0.2–2.0
95th percentile	none–7.3	3.0–18.1	4.1–14.2	3.2–10.0	1.7–6.8	0.9–6.6
Expected typical use level exposure assessment scenario						
Mean	0.03–0.5	0.1–1.7	0.2–1.5	0.2–0.7	0.1–0.5	0.1–0.5
95th percentile	none–2.4	0.6–5.0	0.9–4.5	0.6–2.2	0.4–1.7	0.3–1.5

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

(a): The number of surveys for infants is different compared 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 0.01 mg/kg bw per day in infants to 5.1 mg/kg bw per day in toddlers. The 95th percentile of exposure to the Primary Product ranged from no dietary exposure in infants to 18.1 mg/kg bw per day in toddlers.

At the expected typical use levels, the mean exposure ranged from 0.003 mg/kg bw per day in infants to 1.7 mg/kg bw per day in toddlers, and the 95th percentile of exposure from no dietary exposure in infants to 5.0 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:

- Salami-type sausage belonging to FC 08.3.1.
- Bacon belonging to FC 08.3.1.
- Chorizo and similar 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.

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 of exposure estimates from FAIM and DietEx

The Primary Product is requested to be authorised in 12 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. 5.1 and 18.1 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 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 intraspecies 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 (BMDL10)	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 24 batches of the Primary Product were reported (Table 3). The applicant proposed maximum limits for these toxic elements, as presented in Table 7. However, for cadmium and lead, the Panel noted that the proposed specification limits are lower than their highest measured values (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 and Pb multiplied by a factor of 5 by the Panel, and the LOQs multiplied by a factor of 10 by the Panel for As and Hg; 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

Exposure to SmokEz C-10 (mg/kg bw per day)	(i) Considering the presence of toxic elements at the current EU specifications limits for SmokEz C-10			
	MOE for As at 3 mg/kg	% of the TWI for Cd at 1 mg/kg	MOE for Pb at 5 mg/kg	% of the TWI for Hg at 1 mg/kg
5.1[(a)]	19.6–523	1.4	19.6	0.9
18.1[(b)]	5.5–147	5.1	5.5	3.2
	(ii) Considering the presence of toxic elements at the highest measured value for Cd and Pb multiplied by a factor of 5, and the LOQs multiplied by a factor of 10 by the Panel for As and Hg			
	MOE for As at 1 mg/kg	% of the TWI for Cd at 0.3 mg/kg	MOE for Pb at 2.1 mg/kg	% of the TWI for Hg at 0.05 mg/kg
5.1[(a)]	58.8–1,569	0.4	46.7	0.04
18.1[(b)]	16.6–442	1.5	13.2	0.2

(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 (Table 13). 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 and Pb multiplied by a factor of 5 and the LOQs multiplied by a factor of 10 for As and Hg (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 (Table 13). In this scenario, the presence of the other toxic elements in the Primary Product does 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 24 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 24 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

Exposure to SmokEz C-10 (mg/kg bw per day)	MOE for PAH4
	(i) Considering the presence of PAH4 at the sum of the regulatory limits for benzo[a]pyrene and benzo[a]anthracene in SmokEz C-10 (30 μg/kg)
5.1[(a)]	2.22×10^6
18.1[(b)]	6.26×10^5
	(ii) Considering the presence of PAH4 at their maximum reported level in SmokEz C-10 (2.0 μg/kg)
5.1[(a)]	3.33×10^7
18.1[(b)]	9.39×10^6

(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. 9,000 mg/kg food (Table 9), and the maximum reported level of PAH4 (i.e. the sum of their LOQs) in the Primary Product, i.e. 2.0 μg/kg, the concentration of PAH4 in food would be 0.018 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 48 identified and quantified components of SmokEz C-10 (SF-005) 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).

Of the 48 identified and quantified components, the applicant reported that 31 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.

[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.

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 seven further components, the applicant's conclusions were based on literature search, whereas the remaining nine data-poor substances were assessed by means of read-across (grouping) considerations 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 six 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. 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;

[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/

- Protein binding alerts for chromosomal aberration by OASIS.
- *In vitro* mutagenicity (Ames test) alerts by Istituto Superiore di Sanità (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/(Q)SAR assessment as described above, a risk-based analysis was subsequently applied by the applicant based on a margin of exposure approach. According to the applicant, genotoxicity data can be amendable to dose–response modelling like other toxicological endpoints (Johnson et al., 2014); therefore, 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). Margins of exposure (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 48 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 39 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 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 and 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(*5H*)-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 five components, i.e. hydroxyacetaldehyde (CAS No. 141-46-8), acetaldehyde (CAS No. 75-07-0), formaldehyde (CAS No. 50-00-0), glyoxal (CAS No. 107-22-2) 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(*5H*)-one and benzene-1,2-diol and the potential concern for genotoxicity for the five components listed in (v) 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).

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. All exposure estimates were at least a factor of 64 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 five 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 bw per day to seven individual components 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 13)

CAS No.	Chemical Name	Average content in the Primary Product (wt%)	Exposure	Infants (12 weeks to 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)	0.4	Mean	0.0–8.0	2.0–20.4	3.6–18.8	2.8–14.0	1.6–7.2	0.8–8.0	2.896×10^4
			95th percentile	none–29.2	12.0–72.4	16.4–56.8	12.8–40.0	6.8–27.2	3.6–26.4	
497-23-4	furan-2(5*H*)-one (2(5*H*)furanone)	0.3	Mean	0.0–6.0	1.5–15.3	2.7–14.1	2.1–10.5	1.2–5.4	0.6–6.0	2.172×10^4
			95th percentile	none–21.9	9.0–54.3	12.3–42.6	9.6–30.0	5.1–20.4	2.7–19.8	
Components for which a potential concern for genotoxicity is identified										
141-46-8	hydroxyacetaldehyde	1.1	Mean	0.0–22.0	5.5–56.1	9.9–51.7	7.7–38.5	4.4–19.8	2.2–22.0	7.964×10^4
			95th percentile	none – 80.3	33.0–199.1	45.1–156.2	35.2–110.0	18.7–74.8	9.9–72.6	
75-07-0	acetaldehyde	0.3	Mean	0.0–6.0	1.5–15.3	2.7–14.1	2.1–10.5	1.2–5.4	0.6–6.0	2.172×10^4
			95th percentile	none–21.9	9.0–54.3	12.3–42.6	9.6–30.0	5.1–20.4	2.7–19.8	
50-00-0	formaldehyde	0.3	Mean	0.0–6.0	1.5–15.3	2.7–14.1	2.1–10.5	1.2–5.4	0.6–6.0	2.172×10^4
			95th percentile	none–21.9	9.0–54.3	12.3–42.6	9.6–30.0	5.1–20.4	2.7–19.8	
107-22-2	glyoxal	0.1	Mean	0.0–0.2	0.1–0.5	0.1–0.5	0.1–0.4	0.0–0.2	0.0–0.2	720
			95th percentile	none–0.7	0.3–1.8	0.4–1.4	0.3–1.0	0.2–0.7	0.1–0.7	
107-02-8	2-propenal (acrolein)	9.1×10^{-4}	Mean	0.00–0.02	0.00–0.05	0.01–0.04	0.01–0.03	0.00–0.02	0.00–0.02	64
			95th percentile	none–0.07	0.03–0.16	0.04–0.13	0.03–0.09	0.02–0.06	0.01–0.06	

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 (except the *in vivo* rat liver unscheduled DNA synthesis (UDS) assay), 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 (WARF Institute, 1977), an *in vitro* mammalian cell gene mutation assay in mouse lymphoma cells (TNO, 2005a), an *in vitro* mammalian chromosomal aberration test (TNO, 2005b) and an *in vivo* MN assay in mouse bone marrow (TNO, 2005c).

The evaluation of these studies as described in the scientific opinion 'Safety of smoke flavour Primary Product – SmokEz C-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 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. 06110455) fell within the reported range of batch-to-batch variability and could be 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 are 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 the EFSA CEF panel opinion (EFSA CEF panel, 2009)

3.4.2.1.1. Bacterial reverse mutation test (WARF Institute, 1977)

'The non-GLP assay for gene mutation in bacterial cells was carried out in 1977 on a material described as Carsol (Liquid Smoke), C-10. It used Salmonella typhimurium strains TA 1535, TA1537, TA 1538, TA98 and TA 100 at stated concentrations of 156, 313, 425 or 1250 μg/plate, in the presence or absence of a metabolic activating system and with appropriate positive controls. The material was toxic at levels of 2500 μg/plate and above in the absence of S9 and at 5000 μg/plate in the presence of S9. There was no evidence of an increased number of revertants at any of the non-toxic dose levels in any of the bacterial strains tested. Positive controls gave the expected responses. However, only summary tables of results were provided, containing no experimental details and the test is considered to be of limited validity'. (EFSA CEF Panel, 2009).

'SmokeEz C-10 showed negative results in a S. typhimurium reverse mutation assay in strains TA1535, TA1537, TA1538, TA98 and TA100, both in the absence and presence of S9. The Panel noted that this non-GLP study, carried out in 1977, did not comply with current test guidelines, but did not consider that it was necessary to request the applicant to repeat the study, given that the other two in vitro studies submitted on SmokEz C-10 gave clearly positive results'. (EFSA CEF Panel, 2009).

The FAF Panel agreed with the conclusions of the former evaluation that the results were negative in the tested strains. Nevertheless, the Panel noted some weaknesses in the experimental design. The set of bacterial strains used was incomplete if compared with the current OECD recommendations, in particular S. typhimurium TA102 or *E. coli* WP2 uvrA were not tested. Only two plates per experimental point were used. The Panel considered the study as reliable with restrictions and the results of limited relevance.

3.4.2.1.2. In vitro *mammalian cell gene mutation assay in mouse lymphoma cells (TNO, 2005a)*

'SmokEz C-10 Liquid was tested for mutagenic potential in an in vitro mammalian cell mutation assay (OECD 476) using mouse lymphoma L5178Y tk+/− cells. Two independent assays were carried out. In the first assay, SmokEz C-10 was tested in the dose range 6.1 to 300 μg/ml in the absence of S9-mix and using a 24-hour exposure period, while in the presence of S9 the dose range used was 6.1 to 1250 μg/ml, with a 4-h exposure period. Several lower test concentrations were also used but the cultures were discarded because they were not needed for analytical purposes. In the second assay SmokEz C-10 was tested in the dose range 3.0 to 300 μg/ml in the absence of S9-mix but using a 4-h exposure period. In the presence of S9, a dose range of 100 to 300 μg/ml was used, with smaller intervals between the concentrations, duplicate cultures at each test concentration and an exposure time of 4 h. A solvent control, dimethyl sulfoxide (DMSO), and positive controls (methyl methane sulphonate (MMS) and 3-methyl-cholanthrene (MCA) in the absence and presence of metabolic activation, respectively) were also included in both assays.

The highest concentrations evaluated for mutagenicity were 150 μg/ml in the absence of S9 and 350 μg/ml in the presence of S9, due to the cytotoxicity of SmokEz C-10. In the absence of S9-mix, the relative total growth (RTG) compared with vehicle control cultures was decreased at and above a concentration of 72 μg/ml (RTG 85% in first assay and 74% in second assay), while in the presence of S9-mix, the RTG was decreased at and above 300 μg/ml in the first assay (RTG 28%), and at and above 150 μg/ml in the second assay (RTG 79%). Concentration-related and reproducible positive responses in mutant frequency were observed at concentrations of and above 72 μg/ml in the absence of S9 and 210 μg/ml in the presence of S9. The criterion for a positive response was an increase in induced mutant frequency above concurrent control levels. At concentrations causing a positive response in mutant frequency, relatively more small than large colonies were formed.

It is concluded that SmokEz C-10 demonstrated both mutagenic and clastogenic potential in this in vitro mammalian cell mutation assay'. (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 (2016a). The study was considered reliable without restriction and the results of high relevance.

3.4.2.1.3. In vitro *mammalian chromosomal aberration test (TNO, 2005b)*

'SmokEz C-10 was examined for its potential to induce structural chromosomal aberrations in Chinese Hamster Ovary (CHO) cells (OECD 473), in both the absence and presence of S9 mix. Two independent assays were conducted. The first assay used test concentrations of 39, 78 and 156 μg/ml in the absence of S9 and 156, 313 and 625 μg/ml in the presence of S9. In both cases cells were treated for 4 hours and harvested after 18 hours. In the second assay in the absence of S9 two treatment regimens were used involving (i) test concentrations of 150, 200 and 300 μg/ml, a 4-hour treatment period and harvesting at 18 h, and (ii) test concentrations of 75, 100 and 150 μg/ml for a continuous 18-hour treatment period. In the presence of S9, test concentrations of 300, 400 and 500 μg/ml were used, cells were treated for 4 hours and harvested after 18h.

In the absence of S9, SmokEz C-10 caused a statistically significant ($p < 0.001$) increase in chromosomal aberrations when compared to the solvent control, at 156 μg/ml in the first assay (the highest concentration analysed, causing 50% inhibition of mitotic index and a mean incidence of cells showing chromosome aberrations of 14.5%), at 200 and 300 μg/ml following a 4 h exposure in the second assay (74% and 82% inhibition of mitotic index and 12.5% and 15% aberrant cells, respectively),[16] *and in the continuous treatment group at all concentrations analysed (75, 100 and 150 μg/ml, showing 8%, 9.5% and 20.5% aberrant cells, respectively). In the presence of S9 there was also a statistically significant ($p < 0.001$) increase in the number of aberrant cells, occurring in the first assay at 625 μg/ml (the highest concentration analysed, causing 60% inhibition of mitotic index and 44% aberrant cells) and in the second assay at the two highest concentrations analysed (400 and 500 μg/ml, causing 47% and 62% inhibition of mitotic index and 3% and 10% aberrant cells, respectively).*[17] *While SmokEz C-10 was cytotoxic in this test system, the clastogenic effect was*

[16] The FAF Panel noted that the correct data for % inhibition of mitotic indexes to be considered are 26% and 18%.
[17] The FAF Panel noted that the correct percentages of aberrant cells were 10% and 17.5%.

evident within acceptable toxicity levels. SmokEz C-10 showed evidence of a positive, dose-related clastogenic effect activity 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 metaphases/concentration instead of 300 were scored. Furthermore, no historical ranges for positive controls were reported. Therefore, the Panel considered the study reliable with restrictions and results of limited relevance.

3.4.2.1.4. In vivo *bone marrow mouse micronucleus test (TNO, 2005c)*

'SmokEz C-10 was examined for its genotoxic potential in an in vivo bone marrow mouse micronucleus test in male Charles River CD-1 mice (OECD 474). The test was carried out in male mice only, since in the opinion of the applicant the sub chronic toxicity study in rats carried out at approximate intakes of 2700 mg/kg had revealed no sex differences in toxicity. Group size was 5, and animals received 2 doses of 2000 mg/kg bw SmokEz C-10 in saline by gavage, the doses being given 24 hours apart. A positive control group received a single dose of 0.75 mg/kg bw mitomycin C intraperitoneally, negative control mice received saline alone. Animals were killed 24 hours after the second treatment. No evidence of clinical toxicity was seen in SmokEz C-10-treated animals, and there was no change in NCE/PCE ratio. There was no increase in the frequency of micronucleated polychromatic erythrocytes (MCPE[18]) in male mice at 24 hours after treatment with SmokEz C-10 compared to the vehicle control, while the positive control showed the anticipated increases in MCPE'. (EFSA CEF Panel, 2009).

The FAF 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 and 489 (OECD, 2016c,d), 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.[19] Based on these considerations on the testing of chemical mixtures and considering that the dose of 2,000 mg/kg bw was selected based on a subchronic study in rats, the Panel concluded that the top dose applied could have been higher. However, 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. In addition, no historical control data were provided. 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*

'An in vivo rat liver unscheduled DNA synthesis test was also performed with SmokEz C-10 (batch no. C-10-01217120). DNA repair in hepatocytes was measured following administration by gavage of 2000 mg/kg bw SmokEz C-10 in phosphate-buffered saline (PBS) to 6 male Wistar rats (Crl:[WI] WU

[18] MCPE is an erroneous abbreviation; it should read MNPCE.

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

BR). The negative control group were dosed with PBS alone, while positive control groups (n=2) received either 2-acetylaminofluorene (late sampling period) or N-nitrosodimethylamine (early sampling period). Hepatocytes were isolated at 2-4 h and 12-16 h after exposure, and unscheduled DNA synthesis was measured by autoradiography, following incubation of the hepatocyte cultures with [methyl-^{3}H]-thymidine. SmokEz C-10 did not cause an increase in net nuclear grain count at either sampling time, while positive controls gave expected results. It can be concluded that under the conditions of this study, SmokEz C-10 does not induce unscheduled DNA synthesis in the rat liver'. (EFSA CEF Panel, 2009).

The study report on the *in vivo* UDS assay was not submitted in the new dossier. 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 Salmonella Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 to assess the mutagenicity of SmokEz C-10 (batch no. 06110455), 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 practice (GLP) principles.

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

In Experiment 1, SmokEz C-10 was tested at seven concentrations ranging from 5 to 5,000 μg/plate, and in Experiment 2 at seven concentrations ranging from 160 to 5,000 μg/plate for all strains. In both experiments, positive control chemicals both with and without S9-mix induced significant increases in revertant colony numbers and mean revertant colony numbers for the vehicle controls were within the respective historical control ranges.

No precipitation nor toxicity was reported in any experimental condition.

A twofold increase in the revertant colonies number, over the range of the historical control values, was reported only in experiment 2 (preincubation method) in strain TA100 in the presence of metabolic activation, at the two highest concentrations. No other reproducible and biologically relevant effect was observed in the other bacterial strains.

The Panel considered this result an indication of weak mutagenic activity. The Panel considered the study as reliable without restrictions and the 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 SmokEz C-10 (batch no. 06110455) (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-finder 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 SmokEz C-10 with 12 concentrations ranging from 50 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 50 to 400 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: 100, 230, 320 and 350 μg/mL (cytotoxicity of 9%, 25%, 35% and 54%, respectively). The mean frequency of binucleated cells with micronuclei (MNBN) for the two middle concentrations (0.90% and 1.15% at 230 and 320 μg/mL, respectively) were statistically significantly increased compared to the vehicle control (0.48%) and the increases were concentration-related (trend test: $p < 0.01$). Furthermore, due to elevated MNBN frequencies in one culture, the mean MNBN frequencies for all but the lowest concentration exceeded (i.e. 0.9%, 1.15% and 0.75% at 230, 320 and 350 μg/mL, respectively) 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: 100, 260, 420 and 500 μg/mL (cytotoxicity of 3%, 19%, 39% and 55%, respectively). The mean frequency of MNBN cells at the highest two concentrations (2.40% and 4.50% at 420 and 500 μg/mL, respectively) were statistically significantly increased compared to the vehicle control (0.40%) and the increases were concentration-related (trend test: $p < 0.001$). Furthermore, the mean MNBN frequencies at the two highest concentrations also 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: 150, 240 and 280 μg/mL (cytotoxicity of 10%, 33% and 53%, respectively). The mean frequencies of MNBN cells (4.00%, 6.80% and 10.70%, respectively) were statistically significantly increased compared to the vehicle control (0.48%) and the increases 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, SmokEz C-10 induced statistically significant increases in the mean frequency of MNBN cells compared to vehicle controls and also concentration-dependent trends. Therefore, the authors of this study concluded that SmokEz C-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*

SmokEz C-10 (batch no. 06110455) 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: male groups received 3,500, 5,000 and 7,000 mg/kg bw per day and female groups received 2,500 and 3,500 mg/kg bw per day. In males, all animals in the top dose group were found dead on day 2 and in the mid-dose group two animals lost weight (−0.3 and − 2.0%). Transient effects observed in males included reduced activity (3,500 and 7,000 mg/kg bw per day) and mouth rubbing (3,500 mg/kg bw per day). In females, at the top dose, one animal was found dead on day 2 and one of the surviving animals lost weight (−2.2%); in the mid-dose group all animals lost weight (−0.5 to −1.6%). Transient effects observed included reduced activity and raised hair (3,500 mg/kg bw per day). Therefore, 5,000 mg/kg bw per day was considered the maximum tolerated dose (MTD) for males and 2,500 mg/kg bw per day was considered the MTD for females. The MTD was used as the highest dose level in the main study. As there was a twofold difference in the MTD for male and female rats, the main experiment was performed in both sexes. At all doses,

polychromatic erythrocytes (PCE)/normochromatic erythrocytes (NCE) ratios showed no evidence of bone marrow toxicity.

In the main study, groups of male Han Wistar rats (six per group) were treated via oral gavage with SmokEz C-10 at doses of 0 (vehicle control: deionised water), 1,250, 2,500 or 5,000 mg/kg bw per day; female rats (six per group) received 0, 625, 1,250 or 2,500 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 male and three female rats).

In two animals from the top dose male group, a mild decrease in activity, also accompanied with piloerection in one animal, was observed on 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) for both male and female groups were consistent with the laboratory's respective historical vehicle control (within 95% reference range) data. Mean positive control data for both male and female groups resulted in a statistically significant increase in MNPCE compared to the concurrent vehicle control, which was consistent with the laboratory's respective historical positive control (within 95% reference range) data.

In all dosed groups of male and female rats treated with SmokEz C-10, there were no statistically significant increases in MNPCE mean frequency compared to the vehicle controls and frequencies were also within the laboratory's respective 95% historical vehicle control ranges. Individual frequencies of MNPCE for all treated animals were consistent with historical vehicle control data with the exception of one male animal in the low-dose group and another in the mid-dose group that had mean frequencies of MNPCE 0% and also one female animal in the top-dose group.

The PCE/NCE ratios in males and females were not affected by treatment with SmokEz C-10 and they also fell within the laboratory's respective 95% historical vehicle control ranges.

To demonstrate bone marrow exposure of rats treated with SmokEz C-10, a plasma analysis of a satellite group of animals 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 2,6-dimethoxyphenol in plasma and confirmed that animals were systemically exposed to SmokEz C-10.

The study authors concluded that SmokEz C-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.[20]

Furthermore, the Panel noted that the PCE/NCE ratios were not affected by treatment with SmokEz C-10 and that the mild clinical signs observed in male 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*

SmokEz C-10 (batch no. 06110455) was tested in a 14-day dose range-finding (DRF) (non-GLP) study in Muta™Mouse (CD_2-lacZ80/HazfBR), via the diet, in order to determine the MTD and dose

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

levels for the transgenic rodent (TGR) gene mutation assay using the same rodent strain (Labcorp, 2022c). Bioanalytical analyses were also performed as part of this study.

SmokEz C-10 was administered via the diet (vehicle control: 5 KB3 (5LF2 EU) Rodent Diet) ad libitum to groups of Muta™Mouse animals (three animals per sex per group). 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 4,302, 5,766 and 8,304 mg/kg bw per day in males, and 5,642, 7,839 and 9,912 mg/kg bw per day in females.

In all groups receiving the test item, there were no mortalities, no clinical signs of toxicity, no notable differences in body weight gains (with the exception of one male in the 35,000 mg/kg diet group and one male and one female in the 50,000 mg/kg diet group which lost body weight), organ weights (liver and glandular stomach) or food consumption.

For the bioanalyses, a method was developed for the extraction of 2,6-dimethoxyphenol, a marker analyte, from mouse plasma and subsequent analysis using LC–MS/MS (Labcorp, 2022d). Mean plasma concentrations of 2,6-dimethoxyphenol ranged from less than 5 to 39.5 ng/mL (limit of quantitation 5 ng/mL). The highest concentrations of 2,6-dimethoxyphenol were present on Day 14 for both males and females at all dietary concentrations.

The maximum tolerated concentration in feed for both males and females was considered to be 50,000 mg/kg diet for a 28-day dosing period. This concentration was also considered by the authors to be the maximum feasible concentration, in order to avoid nutritional disturbances. As no difference in toxicity was observed between male and female animals, only male animals were tested in the main study.

In the *in vivo* gene mutation assay in Muta™Mouse (*lacZ/GalE*), SmokEz C-10 (batch no. 06110455) was administered via the diet to three groups of male transgenic CD_2-LacZ80/HazfBR mice (seven animals per group) for 28 consecutive days (Labcorp, 2022b). This study was performed according to OECD TG 488 (OECD, 2020b) and in compliance with GLP. Animals received dietary concentrations of 12,500, 25,000 or 50,000 mg/kg diet corresponding to overall mean dose levels of 2,002, 4,140 and 8,639 mg/kg bw per day. 5 KB3 (5LF2 EU) Rodent Diet was used as the vehicle control (seven 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 seven animals administered ethyl-N-nitrosourea (ENU) at a dose of 50 mg/kg bw from a previous study.

Separate satellite groups of six male Muta™Mice were also included within the study design 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 notable differences in body weight gains and organ weights (liver and glandular stomach) or food consumption.

Liver and glandular stomach samples from seven animals per dose groups and positive and vehicle control groups were processed for DNA isolation.

For each DNA sample from vehicle controls 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 required minimum of 125,000 plaques). For DNA samples from positive control-treated animals, the number of plaques from 1 to 14 packagings was greater than 125,000, with three exceptions where data from 1 to 3 packaging(s) was reported and was 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 test laboratory's historical range. There was a single instance for the liver and three instances for the glandular stomach of individual animal results falling below the respective ranges. However, the study authors 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.

Treatment with SmokEz C-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 (22.63–117.97 $\times$ 10^6 plaque forming units (pfu)), with the exception of data for one animal from the low dose group (22.05 $\times$ 10^6 pfu) and one animal from the mid-dose group (22.04 $\times$ 10^6 pfu) that fell only slightly below this range, but were within the historical vehicle control observed range (15.65–382.28 $\times$ 10^6 pfu). There was no dose–response relationship.

In the glandular stomach, no significant increases in mutation frequency were observed, with individual animal and group mean mutation frequency falling within the test laboratory's historical

vehicle control observed range (29.04–119.50 $\times$ 10^6 pfu, based on only 25 animals). However, there were exceptions for a total of six animals from all treated groups (ranging 20.26 $\times$ 10^6 pfu – 28.10 $\times$ 10^6 pfu), which fell below the laboratory's historical vehicle control observed range. The study authors considered the results close enough to existing ranges to consider the data acceptable. There was no dose–response relationship.

The study authors concluded that in this *in vivo* gene mutation assay in Muta™Mouse (lacZ/GalE), SmokEz C-10 did not induce increases 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 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 SmokEz C-10, 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 a mixture of sawdust comprising hard maple (*Acer saccharum*) (25–60%), white oak (*Quercus alba*) (10–40%), hickory (*Carya ovata*) (10–25%) and minor quantities of white/black ash (*Fraxinus americana*) (0–15%), birch (*Betula papyrifera* and *Betula alleghanisensis*) (0–15%), beech (*Fagus grandifolia*) (0–15%) and cherry (*Prunus serotina*) (0–15%).

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 3.1.2.4.2), 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) 627/2006).

The applicant reported data on the batch-to-batch variability of 300 batches of the Primary Product. The observed relative standard deviations for the monitored chemical parameters were on average $<$ 16% (see Table 6). In addition, the applicant performed statistical analyses for 20 selected compounds, in 23 batches produced from July 2020 to July 2022. The observed relative standard deviations were on average $<$ 19%. 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 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 for cadmium and lead for which the proposed specification limits are lower than their highest measured values of 0.06 and 0.41 mg/kg for Cd and Pb, respectively (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 and Pb multiplied by a factor of 5 and the LOQs multiplied by a factor of 10 for As and Hg (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 9.39×10^6 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 12 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 0.01 mg/kg bw per day in infants to 5.1 mg/kg bw per day in toddlers (Table 12). The 95th percentiles DietEx exposure estimates ranged from no dietary exposure in infants to 18.1 mg/kg bw per day in toddlers. At the expected typical use levels, the mean DietEx dietary exposure estimates ranged from 0.03 mg/kg bw per day in infants to 1.7 mg/kg bw per day in toddlers; the 95th percentile DietEx exposure estimates ranged from no dietary exposure in infants to 5.0 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 48 individual components of the Primary Product, the Panel considered that:

i) for 41 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(*5H*)-one and benzene-1,2-diol, which are present in the Primary Product at average concentrations of 0.3 wt% and 0.4 wt%, respectively;
iii) for five 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 seven components listed in (ii) and (iii) are given and discussed in Appendix B.

Regarding the two components furan-2(*5H*)-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(*5H*)-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,b,c). From these EFSA opinions, no evidence is available to prove that furan-2(*5H*)-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, 2016) 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(*5H*)-one and benzene-1,2-diol and the potential concern for genotoxicity for the five components mentioned above in (iii) 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). 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 all the seven 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 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 these seven components. 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 showed a weak mutagenic activity in a bacterial reverse mutation assay and induced gene mutations in mammalian cells *in vitro*. 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.

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(*5H*)-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 five 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 SmokEz C-10 (SF-005).

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-005. 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 cannot 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 nanospecific considerations.

The Panel identified a potential concern for genotoxicity for five components in the Primary Product as well as for the unidentified fraction of the mixture. More importantly, the Primary Product contains furan-2(*5H*)-one and benzene-1,2-diol, two known *in vivo* genotoxic substances via the oral route. Considering that the exposure estimates for furan-2(*5H*)-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 SmokEz C-10 (SF-005) raises concern with respect to genotoxicity.

6. Documentation as provided to EFSA

1) Dossier "Application for renewal of an already authorised smoke flavouring – SmokEz C-10". Dossier number: SFL-2022-7011. June 2022. Submitted by Kerry Inc.[21]
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) Labcorp, 2021a. SMOKEZ C-10 (SF-005): Bacterial Reverse Mutation Assay. Labcorp Early Development Laboratories Ltd. Study No. 8447541, September 2021. Unpublished study report submitted by Kerry Inc.
5) Labcorp, 2021b. SmokEz C-10 (SF-005): *In Vitro* Human Lymphocyte Micronucleus Assay. Labcorp Early Development Laboratories Ltd. Study No. 8447542, September 2021. Unpublished study report submitted by Kerry Inc.
6) Labcorp, 2021c. Smokez C-10 (SF-005) [Item code C10BT]: Validation of an Analytical Method and Formulation Accuracy, Homogeneity and Stability in Liquid Vehicle and Diet. Labcorp Early Development Laboratories Ltd. Study No. 8447554, August 2021. Unpublished study report submitted by Kerry Inc.
7) Labcorp, 2022a. SmokEz C-10 (SF-005): Rat Bone Marrow Micronucleus Assay. Labcorp Early Development Laboratories Ltd. Study No. 8447544, May 2022. Unpublished study report submitted by Kerry Inc.

[21] 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-2022-7011

8) Labcorp, 2022b. SmokEz C-10 (SF-005): Transgenic Gene Mutation Assay in Muta™Mice. Labcorp Early Development Laboratories Ltd. Study No. 8476535, August 2022. Unpublished study report submitted by Kerry Inc.
9) Labcorp, 2022c. SmokEz C-10 (SF-005): Exploratory 14-Day Dose Range-Finding Study in Muta™Mice. Labcorp Early Development Laboratories Ltd. Study No. 8447545, April 2022. Unpublished study report submitted by Kerry Inc.
10) Labcorp, 2022d. 2,6-Dimethoxyphenol: Validation of Methodology for the Determination of Residues in Mouse Plasma. Labcorp Early Development Laboratories Ltd. Study No. 8447539, March 2022. Unpublished study report submitted by Kerry Inc.
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12) TNO, 2005b. Chromosomal aberration test with Smokez C-10 in cultured Chinese Hamster Ovary (CHO) cells. TNO Nutrition and Food Research Institute, The Netherlands. Study No. 5602/16 January 2005. Unpublished study report submitted by Kerry Inc
13) TNO, 2005c. Micronucleus test in bone marrow cells of mice treated with Smokez C-10. TNO Nutrition and Food Research Institute, The Netherlands. Study No. 6204/01 June 2005. Unpublished study report submitted by Kerry Inc.
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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
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
IQ	intelligent quotient
ip	intraperitoneal
ISS	Istituto Superiore di Sanità
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOQ	limit of quantification
ML	maximum level
MN	micronucleus
MNPCE	micronucleated polychromatic erythrocytes
MOE	margin of exposure
MTD	maximum tolerated dose
NCE	normochromatic erythrocytes
NOAELs	no observed adverse effect levels
OECD	Organisation for Economic Co-operation and Development
P95	95th percentile
PAHs	polycyclic aromatic hydrocarbons
PCE	polychromatic erythrocytes
QSAR	quantitative structure–activity relationship
ROS	reactive oxygen species
RSD	relative standard deviation
RTG	Relative total growth
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
WT	weight

Appendix A – Full list of identified and quantified constituents of smoke flavouring Primary Product SF-005

Table A.1: Compilation of the 48 identified and quantified volatile constituents in the Primary Product (Documentation provided to EFSA No. 1)

CAS-no.	FL-no	Chemical name[(a)]	Conc. in wt%[(b)]
64-19-7	08.002	Acetic acid	10.2
498-07-7		β-D-glucopyranose, 1,6-anhydro-	2.5
64-18-6	08.001	Formic acid	1.6
141-46-8		acetaldehyde, hydroxy-	1.1
116-09-6	07.169	1-hydroxypropan-2-one (2-propanone, 1-hydroxy-)	1.0
79-09-4	08.003	Propionic acid (propanoic acid)	0.5
120-80-9	04.029	benzene-1,2-diol (catechol)	0.4
80-71-7	07.056[(c)]	3-methylcyclopentan-1,2-dione (2-cyclopenten-1-one, 2-hydroxy-3-methyl-)	0.3
67-56-1		Methanol	0.3
50-00-0		Formaldehyde	0.3
497-23-4	former 10.066[(d)]	2(5*H*)-furanone	0.3
75-07-0	05.001	Acetaldehyde	0.3
107-21-1		Ethylene glycol	0.2
79-20-9	09.023	Methyl acetate (acetic acid, methyl ester)	0.2
91-10-1	04.036	2,6-dimethoxyphenol (phenol, 2,6-dimethoxy-)	0.2
98-01-1	13.018	Furfural	0.2
107-22-2		Glyoxal	0.1
78-98-8	07.001	2-oxopropanal (methylglyoxal)	0.1
108-95-2	04.041	Phenol	0.1
497-09-6		propanal, 2,3-dihydroxy-, (*S*)-	0.1
90-05-1	04.005	2-methoxyphenol (phenol, 2-methoxy-)	0.05
67-64-1	07.050	Acetone	0.05

CAS-no.	FL-no	Chemical name[a]	Conc. in wt%[b]
67-47-0	13.139	5-hydroxymethylfurfuraldehyde (5-hydroxymethylfurfura)	0.04
620-02-0	13.001	5-methylfurfural (2-furancarboxaldehyde, 5-methyl-)	0.03
118-71-8	07.014	Maltol	0.03
95-48-7	04.027	2-methylphenol (phenol, 2-methyl-)	0.03
431-03-8	07.052	Diacetyl (2,3-butanedione)	0.02
106-44-5	04.028	4- methylphenol (*p*-cresol)	0.02
107-18-6		2-propen-1-ol	0.02
107-31-3	09.642	Methyl formate	0.02
120-92-3	07.149	Cyclopentanone	0.009
554-12-1	09.134	Methyl propionate	0.007
105-67-9	04.066	2,4-dimethylphenol (phenol, 2,4-dimethyl-)	0.006
3102-33-8	07.044[e]	pent-3-en-2-one (3-penten-2-one, (*E*)-)	0.004
600-14-6	07.060	pentan-2,3-dione (2,3-pentanedione)	0.004
64-17-5	02.078	Ethanol	0.002
78-93-3	07.053	butan-2-one (2-butanone)	0.002
623-42-7	09.038	Methyl butyrate (butanoic acid, methyl ester)	0.002
1489-69-6		Cyclopropanecarboxaldehyde	0.001
576-26-1	04.042	2,6-dimethylphenol (phenol, 2,6-dimethyl-)	0.001
107-02-8		2-propenal	9.1×10^{-4}
71-23-8	02.002	propan-1-ol (1-propanol)	8.9×10^{-4}
109-87-5	06.074	dimethoxymethane (methylal)	7.5×10^{-4}

CAS-no.	FL-no	Chemical name[a]	Conc. in wt%[b]
814-78-8		3-buten-2-one, 3-methyl-	7.2×10^{-4}
78-94-4		2-butenone [methyl vinyl ketone; 3-buten-2-one]	5.6×10^{-4}
123-72-8	05.003	Butanal	4.6×10^{-4}
109-49-9	07.162	hex-5-en-2-one (5-hexen-2-one)	2.5×10^{-4}
590-86-3	05.006	3-methylbutanal (butanal, 3-methyl-)	9×10^{-5}

(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): [FL-no: 07.056] refers to the mixture of the tautomeric forms of 3-methylcyclopentan-1,2-dione.
(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: 07.044] refers to the mixture of *E/Z* stereoisomers of pent-3-en-2-one.

Appendix B – Genotoxicity data available on 7 individual components for which a (potential) concern for genotoxicity is identified

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

i) furan-2(*5H*)-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) five 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), glyoxal (CAS No. 107-22-2) and 2-propenal (acrolein, CAS No. 107-02-8).

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

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

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

The applicant further assessed the potential risk to furan-2(*5H*)-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 ~ 19,000. The applicant speculated that the MOE for carcinogenicity might be even higher if there were tumour data.

Conclusion: The Panel considered that furan-2(*5H*)-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(*5H*)-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(*5H*)-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[22]). In the evaluation by CoE,[23] 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[24] have been evaluated more recently by ECHA (ECHA, 2016a), leading to a standardised classification for genotoxicity as 'Muta 2' for this substance.[25]

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

[22] 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, 19.07.2000, p. 8–16
[23] https://book.coe.int/en/health-protection-of-the-consumer/2208-chemically-defined-flavouring-substances.html
[24] 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)
[25] https://echa.europa.eu/da/information-on-chemicals/cl-inventory-database/-/discli/details/67398

concern emerges since the exposure to benzene-1,2-diol exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 16).

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

The applicant provided *in silico* 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 also 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 Salmonella 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 Salmonella 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 detoxication 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 at least 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 the 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,b) 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 at least 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. Glyoxal (CAS No. 107-22-2)

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.

Glyoxal was evaluated by the European Commission's Scientific Committee on Consumer Products (SCCP, 2005). Based on the *in vitro* genotoxicity studies evaluated by the SCCP, the Panel noted positive results for gene mutations in bacteria and in some of the studies in mammalian cells. Chromosomal and DNA damage were also observed in mammalian cells. Negative results were observed in an *in vivo* MN study in mice administered via ip injection. In *in vivo* UDS studies, DNA damage was observed in liver and pyloric mucosa of rats (SCCP, 2005).

Conclusions: Based on the experimental data available, glyoxal is genotoxic *in vitro*. The panel considered that *in vivo* studies reported by SCCP (2005) are not sufficient to rule out the potential concern for genotoxicity raised from the *in vitro* data. Therefore, *in vivo* follow-up studies, addressing gene mutations and structural and numerical chromosomal aberrations would be needed to evaluate the genotoxic potential of the substance, since the exposure to glyoxal exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 16).

B.7. 2-Propenal (acrolein) (CAS No. 107-02-8)

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.

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 reactive oxygen species (ROS) 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 (8 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 three 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 the 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, 2020).

The induction of DNA single strand breaks by acrolein, evaluated by alkaline elution and by comet assay, was 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 to clarify the potential genotoxicity of acrolein. Therefore, *in vivo* genotoxicity studies following oral administration would be required. These studies should address gene mutations and structural and numerical chromosomal aberrations at least at the site of contact, since the exposure to acrolein exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 16).

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[26] '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 judgement'.

4. Reliability cannot be evaluated[27] '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:

[26] Klimisch et al. (1997) used the term 'Not reliable' however, 'Reliability Insufficient' was considered more appropriate.
[27] 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 vs. 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 SmokEz C-10 (SF-005) 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
SmokEz C-10	Bacterial Reverse Mutation test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	156–1,250 μg/plate (+/−S9)	Negative	Reliable with restrictions (incomplete battery of bacterial strains, only 2 plates per experiment, limited reporting of the study). Study performed before GLP criteria and OECD TG 471 were established.	High/Limited	WARF Institute (1977)
	In vitro mammalian cell gene mutation test in mouse lymphoma cells	L5178Y TK $^{+/-}$ mouse lymphoma cells	Experiment 1: 6.1–300 μg/mL (24 h, −S9) 6.1–1,250 μg/mL (4 h, +S9) Experiment 2: 3–300 μg/mL (4 h, −S9) 100–350 μg/mL (4 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	TNO (2005a)
	In vitro mammalian chromosomal aberration test	Chinese hamster ovary cells (CHO K-1 cell line)	Experiment 1: 156, 313, 625 μg/mL (4 + 18 h, +S9) 39, 78, 156 μg/mL (4 + 18 h, −S9) Experiment 2: 300, 400, 500 μg/mL (4 + 18 h, +S9) 150, 200, 300 μg/mL (4 + 18 h, −S9) 75, 100, 150 μg/mL (18 h, −S9)	Positive	Reliable with restrictions (only 200 metaphases/concentration instead of 300 were scored; no historical ranges for positive controls were reported). Study performed according to OECD TG 473 and in compliance with GLP	High/Limited	TNO (2005b)

Table D.2: Summary of *in vivo* genotoxicity studies on SmokEz C-10 (SF-005) 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
SmokEz C-10	Micronucleus assay in bone marrow	Charles River CD-1 mice; M Gavage	2,000[(a)]	Inconclusive (negative, but without demonstration of bone marrow exposure)	Reliable with restrictions (higher doses might have been applied; lack of demonstration of bone marrow exposure; historical control data not reported). Study performed according to OECD TG 474 and in compliance with GLP	High/low	TNO (2005c)

M: males.
(a): Dose administered to mice, by gavage, on two successive days with an interval of approximately 24 h. Animals sacrificed 24 h after the second administration.

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

Table E.1: Summary of new *in vitro* genotoxicity studies on SmokEz C-10 (SF-005)

Name	Test system *in vitro*	Test object	Concentrations[(a)] and Test conditions	Result	Reliability/comments	Relevance of test system/ relevance of the result	Reference
SmokEz C-10	Reverse Mutation test	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	Experiment 1: 5–5,000 μg/plate (+/−S9, plate incorporation) Experiment 2: 160–5,000 μg/plate (+/−S9, pre-incubation)	Weak 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	100, 230, 320 and 350 μg/mL (3 + 21 h, −S9) 100, 260, 420 and 500 μg/mL (3 + 21 h, +S9) 150, 240 and 280 μg/mL (24 + 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* micronucleus 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 SmokEz C-10 (SF-005)

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
SmokEz C-10	Micronucleus assay in bone marrow	Han Wistar rats; M & F gavage	1,250, 2,500 and 5,000 (M); 625, 1,250 and 2,500 (F)[(a)]	Inconclusive (negative, but without demonstration of sufficient bone marrow exposure)	Reliable with restrictions (no demonstration of sufficient bone marrow exposure). Study performed according to OECD TG 474 and in compliance with GLP	High/low	Labcorp (2022a)
	Gene mutation assay in liver and glandular stomach	Muta™Mouse (lacZ/ GalE) CD_2-LacZ80/ HazfBR SPF transgenic mice; M diet	2,002, 4,140 and 8,639[(b)]	Negative	Reliable without restrictions. Study performed according to OECD TG 488 and in compliance with GLP	High/high	Labcorp (2022b)

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 12,500, 25,000 and 50,000 mg/kg diet.

Annex A – Exposure assessment results

- Annex A1: Occurrence data per food category considered in FAIM (mg/kg).
- Annex A2: Total estimated exposure of SmokEz C-10 (SF-005) 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 SmokEz C-10 (SF-005) 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 SmokEx C-10 (SF-005) 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 SmokEx C-10 (SF-005) 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 SmokEx C-10 (SF-005) 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 SmokEx C-10 (SF-005) 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.