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Scientific opinion on the renewal of the authorisation of Smoke Concentrate 809045 (SF-003) 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 Smoke Concentrate 809045 (SF-003), 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. Product Smoke Concentrate 809045 is obtained by pyrolysis of beech wood. The Panel concluded that the compositional data provided on the Primary Product are adequate. At the maximum proposed use levels, dietary exposure estimates calculated with DietEx ranged from 0.1 to 1.5 mg/kg body weight (bw) per day at the mean and from 0.2 to 5.2 mg/kg bw per day at the 95th percentile. The Panel concluded that eleven components in the Primary Product raise a potential concern for genotoxicity. In addition, a potential concern for genotoxicity was identified for the unidentified part of the mixture. The Primary Product contains furan-2(5*H*)-one and benzene-1,2-diol, for which a concern for genotoxicity was identified *in vivo* upon oral administration. Considering that the exposure estimates for these two components are above the threshold of toxicological concern (TTC) of 0.0025 μg/kg bw per day for DNA-reactive mutagens and/or carcinogens, the Panel concluded that the Primary Product raises concern with respect to genotoxicity.

Keywords: Smoke Concentrate 809045, SF-003, smoke flavouring Primary Product, genotoxicity, furan-2(5*H*)-one, benzene-1,2-diol

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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 smoke flavouring or any food where such a smoke flavouring is present (in or on) can be placed in the market if the smoke flavouring is not an authorised primary product or is not derived therefrom and if the conditions of use laid down in the authorisation in accordance with this Regulation are not adhered to (Article 4(2) of Regulation (EC) No 2065/2003).

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

The European Commission has received an application for the renewal of the authorisation of the smoke flavouring primary product Smoke Concentrate 809045 (SF-003) 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 Smoke Concentrate 809045 (SF-003), for which a renewal application has been submitted, in accordance with Article 12(1) 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 to Smoke Concentrate 809045 (SF-003). 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 Smoke Concentrate 809045 in 2009 (EFSA CEF Panel, 2009).

Following the safety assessment from EFSA, Smoke Concentrate 809045 was authorised in the European Union and assigned the unique code 'SF-003', 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 Smoke Concentrate 809045 (SF-003) 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, pp. 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, pp. 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 Smoke Concentrate 809045 (SF-003). In accordance with Article 38 of the Regulation (EC) No 178/2002[3] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation and of the Decision of the EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[4] the non-confidential version of the dossier is published on Open.EFSA.[5]

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the application from 1 February to 22 February 2023, for which no comments were received.

Additional information was sought from the applicant during the assessment process in response to requests from EFSA sent on 11 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 No. 4). 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 Smoke Concentrate 809045 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 G1 therein (EFSA FAF Panel, 2021). Standard uncertainties were not discussed in detail in the present assessment. In case of the presence of non-standard uncertainties, these were 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 Smoke Concentrate 809045 is only beech wood (*Fagus sylvatica* L.). According to the applicant, the wood is obtained from untreated natural hardwood and is free from pesticides (Documentation provided to EFSA No. 1). The certificates of quality proving that the raw material complies with the specifications provided by the suppliers were submitted.

[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, pp. 1–48.

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

[5] The non-confidential version of the dossier, following EFSA's assessment of the applicant's confidentiality requests, is published on Open.EFSA and is available at the following link: https://open.efsa.europa.eu/dossier/SFL-2021-2352.

3.1.1.2. Method of manufacture of the Primary Product

The production of the Primary Product comprises the following steps:

1) Smoke generation: The dried wood chips are smouldered in a smoke generator under defined conditions.
2) Condensation and absorption of smoke: The smoke is passed through a condenser and subsequently absorbed in a water/ethanol mixture, and the formed wood tar is then discarded.
3) Further processing: The liquid smoke is treated with activated charcoal to reduce the levels of polycyclic aromatic hydrocarbons (PAHs). The charcoal is then removed by filtration, and the Primary Product is obtained after removing the residual solvents by distillation.

The applicant submitted a description of the manufacturing process, with information on the drying step 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 product is Smoke Concentrate 809045.

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

The applicant indicated that the smoke flavouring Smoke Concentrate 809045 has not been evaluated by regulatory bodies other than EFSA (Documentation provided to EFSA no. 1).

Regarding the existing authorisations in non-EU countries, the applicant stated that Smoke Concentrate 809045 is currently authorised in the United Kingdom (see Documentation provided to EFSA No. 4).

3.1.2.3. Description of the physical state and sensory characteristics

The Primary Product is a brown viscous liquid with a characteristic odour of freshly generated smoke and has an average density (at 4 °C) of 1,275 g/L (n = 12). The pH ranges from 2.8 to 3.0, the refraction index ranges from 1.51 to 1.52, the coefficient of extinction (at 400 nm) ranges from 1.9 to 2.4 and the flash point is > 100 °C (Documentation provided to EFSA No. 2).

3.1.2.4. Chemical composition of the Primary Product

The compositional data provided by the applicant for two batches of the Primary Product, in response to the EFSA requests for additional information, are summarised in Table 1 (Documentation provided to EFSA No. 2). The applicant submitted information for two batches (no.: 10300233 and 10300236), for which investigations of the non-volatile fraction (Section 3.1.2.4.3) have been performed. Taking into account the data provided on the variability of individual volatile constituents (Section 3.1.2.6; Table 7), the Panel noted that these two batches fell within the range of batch-to-batch variability reported for the other 10 investigated batches and considered them as representative of the Primary Product.

Table 1: Overview on the compositional data provided for two batches of the Primary Product (Documentation provided to EFSA No. 2)

Batch no.	Density (g/L)	Total volatiles (wt%)	Identified volatiles (wt%)	Unidentified volatiles (wt%)	Total non-volatiles (wt%)(1)	Identified non-volatiles (wt%)	Unidentified non-volatiles (wt%)	Water (wt%)	Solvent-free fraction (wt%)	Ident./quant. proportion of solvent-free fraction (wt%)(2),(a)	Ident./quant. proportion of volatile fraction (wt%)(3),(b)
10300233(#)	1,274	48.1	39.9	8.2	43.7	19.6	24.1	8.2	91.8	64.8	83.0
10300236(#)	1,279	40.1	33.0	7.1	51.3	19.2	32.1	8.6	91.4	57.1	82.3
Average	1,277	44.1	36.5	7.7	47.5	19.4	28.1	8.4	91.6	61.0	82.6

wt: weight.
(1): Calculated as the sum of identified and unidentified non-volatiles (wt%).
(2): Calculated as ((identified volatiles + identified non-volatiles)/solvent-free fraction) × 100.
(3): Calculated as (identified volatiles/total volatiles) × 100.
(#): Batches tested in the toxicological studies.
(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, pp. 3–6.

3.1.2.4.1. Chemical characterisation

The water content of the Primary Product was determined by the Karl Fischer titration method. The applicant also provided data on the contents of the major chemical classes in the Primary Product, i.e. acids, carbonyls and phenols in 12 batches of the Primary Product (Table 2) (Documentation provided to EFSA No. 1).

Table 2: Overview on the compositional data provided for 12 batches of the Primary Product

	Batch no.												Average	SD
	10300221	10300223	10300229	10300231	10300232	10300233(#)	10300234	10300235	10300236(#)	10300237	10300238	10300239		
Acids (wt%) (as acetic acid)	12.3	11.3	10.8	11.2	9.9	10.4	12.1	12.1	12.0	11.4	11.5	12.7	11.5	0.8
Phenols (wt%) (as 2,6-dimethoxyphenol)	0.6	0.6	0.5	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.03
Carbonyls (wt%) (as 2-butanone)	16.5	13.6	16.7	16.9	16.6	16.0	13.0	15.4	14.5	13.6	13.7	15.5	15.2	1.4
Water (wt%)	10.1	8.3	9.2	9.4	9.0	8.2	8.5	12.0	8.9	7.4	8.6	9.0	9.1	1.1

wt: weight; SD: standard deviation.
(#): Batches tested in toxicological studies.

Concentrations of arsenic, cadmium, lead and mercury were determined by inductively coupled plasma–mass spectrometry (ICP–MS) and were submitted to EFSA (Table 3) (Documentation provided to EFSA No. 1).

Table 3: Toxic elements reported for 12 batches of the Primary Product

	Batch no. (mg/kg)												Average (mg/kg)	SD
	10300221	10300223	10300229	10300231	10300232	10300233(#)	10300234	10300235	10300236(#)	10300237	10300238	10300239		
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	–
Cadmium (Cd)	0.06	0.05	0.06	0.05	0.05	0.05	0.04	0.04	0.03	0.03	0.05	0.04	0.05	0.01
Lead (Pb)	0.13	0.10	0.16	0.11	0.12	0.11	0.34	0.28	0.32	0.39	0.32	0.35	0.23	0.11
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	–

SD: standard deviation.
(#): Batches tested in toxicological studies.
(<): This symbol means that the concentration of the toxic element was below the corresponding LOQ.

3.1.2.4.2. Identification and quantification of the volatile fraction

Gas chromatography–mass spectrometry (GC–MS) and gas chromatography–flame ionisation detection (GC–FID) were applied for identification and quantification of the constituents of the volatile fraction of the Primary Product. Individual volatile constituents were considered as identified if their chromatographic (Kovats retention indices) and their mass spectral data were in agreement with those of reference standards. Overall, using this approach, 129 volatile constituents were identified and quantified in the Primary Product (Documentation provided to EFSA No. 1 and 2).

In addition, the applicant reported 14 tentatively identified volatile constituents (Documentation provided to EFSA No. 1). The identification was considered 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 as part of the unidentified fraction.

Regarding the quantifications of volatile constituents, the applicant provided the following sets of data:

(a) Semi-quantifications of the volatile components were based on the area-% determined via GC–FID. The applicant applied relative gas chromatography (GC)-response factors predicted according to a modified version of a mathematical model developed by the International Organization of the Flavor Industry (IOFI) (Cachet et al., 2016). 2,6-dimethoxyphenol (CAS no.: 91-10-1) and dihydro-3-hydroxy-2(3*H*)-furanone (CAS no.: 19444-84-9) were used as reference components for aromatic type and non-aromatic type volatiles, respectively (Documentation provided to EFSA No. 2). The 129 identified volatile constituents were semi-quantified on the basis of this approach in 12 batches of the Primary Product (for batch numbers see Table 2) (Appendix A, Table A.1). The lowest concentration reported by the applicant was 0.001 weight (wt)% for 2,5-dimethyl- pyridine (CAS no.: 589-93-5).

The 20 principal volatile constituents are presented in Table 4. This list also contains acetic acid and formic acid which have been quantified separately via ion chromatography (IC), because the IOFI model, to predict response factors, is not applicable to polar small molecules with a high proportion of oxygen atoms.

(b) For 31 major volatile constituents, the applicant performed quantifications using external calibrations. Depending on the type of constituent, different approaches based on GC–MS/MS, liquid chromatography–mass spectroscopy (LC–MS), IC or GC/MS were applied. Validation parameters of the employed analytical methods were provided. For 16 of the principal volatile constituents, the average concentrations determined via these quantification approaches in 12 batches of the Primary Product are presented in Table 4.

Table 4: Twenty principal volatile constituents of the Primary Product (Documentation provided to EFSA No. 1 and 2)

CAS no.	FL-no	Chemical name[a]	Semi-quantitative values (wt%)[b]	Quantitative values (wt%)[c]	Concentrations (as wt%) in the former application[d]
64-19-7	08.002	Acetic acid	7.6	7.1	5.9
498-07-7	–	β-D-glucopyranose, 1,6-anhydro-	4.2	5.1	5.4
91-10-1	04.036	2,6-dimethoxyphenol (phenol, 2,6-dimethoxy-)	2.0	2.0	1.9
116-09-6	07.169	1-hydroxypropan-2-one (2-propanone, 1-hydroxy)	1.8	0.6	1.2
6638-05-7	04.053	4-methyl-2,6-dimethoxyphenol (phenol, 2,6-dimethoxy,-4-methyl-)	1.3	1.3	1.6
141-46-8	–	Acetaldehyde, hydroxy-	1.2	0.5	1.2
10374-51-3	–	2(3*H*)-furanone, dihydro-5-(hydroxymethyl)-	0.9	0.8	n.d.
107-21-1	–	1,2-ethanediol (ethylene glycol)	0.9	0.2	0.6
16874-33-2	–	2-furancarboxylic acid, tetrahydro-	0.9	< 0.5	n.d.
64-18-6	08.001	Formic acid	0.8	0.9	n.d.
203506-97-2	–	Threo-pentonic acid, 3-deoxy-, γ-lactone	0.8	–[e]	n.d.
19444-84-9	–	2(3*H*)-furanone, dihydro-3-hydroxy-	0.7	–[e]	0.1
765-70-8	07.056[f]	3-methylcyclopentan-1,2-dione (1,2-cyclopentanedione, 3-methyl)	0.7	0.7	n.d.
14059-92-8	04.052	4-ethyl-2,6-dimethoxyphenol (phenol, 4-ethyl-2,6-dimethoxy)	0.7	0.7	0.6
123-76-2	08.023	4-oxovaleric acid (pentanoic acid, 4-oxo-)	0.6	–[e]	n.d.
120-80-9	04.029	Benzene-1,2-diol (1,2-benzenediol)	0.5	1.2	0.6
58534-89-7	–	3(2*H*)-furanone, dihydro-5-(hydroxymethyl)-	0.4	–[e]	n.d.

CAS no.	FL-no	Chemical name[a]	Semi-quantitative values (wt%)[b]	Quantitative values (wt%)[c]	Concentrations (as wt%) in the former application[d]
20675-95-0	04.055[g]	2,6-dimethoxy-4-prop-1-enylphenol (phenol, 2,6-dimethoxy-4-(1*E*)-1-propen-1-yl-)	0.4	0.4	0.4
497-23-4	Former 10.066[h]	Furan-2(5*H*)-one (2(5*H*)furanone)	0.4	0.6	0.4
5058-01-5	–	2*H*-pyran-2-one, tetrahydro-3- hydroxy-	0.3	0.2	n.d.

CAS: Chemical Abstract Service; FL-no: FLAVIS number; wt: weight.
n.d.: not detected in the previous safety evaluation of the Primary Product (EFSA CEF Panel, 2009).
(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): Semi-quantifications based on GC–FID area-%, applying relative GC response factors predicted according to a modified mathematical model developed by IOFI. Values represent means determined by analysis of 12 batches of the Primary Product.
(c): Quantifications based on GC–MS/MS, LC–MS, IC and GC/MS, respectively, using external calibrations. The values presented are average values, calculated from the 12 batches listed in Table 7.
(d): From the data presented in the previous safety evaluation of the Primary Product (EFSA CEF Panel, 2009).
(e): Compound not quantified; only semi-quantitative value available.
(f): [FL-no: 07.056] refers to the mixture of the tautomeric forms of 3-methylcyclopentan-1,2-dione.
(g): [FL-no: 04.055] refers to the mixture of *E/Z* stereoisomers of 2,6-dimethoxy-4-prop-1-enylphenol.
(h): '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.

(c) In addition, quantitative data were provided for seven selected volatile constituents, which the applicant considered as 'toxicologically relevant'. The quantifications were performed by an external laboratory through GC–MS (i.e. time of flight–mass spectroscopy (TOF–MS)) using isotopically labelled standards. Certificates of analyses and a description of the analytical method were provided (Documentation provided to EFSA No. 1). A summary of the results obtained for five production batches of the Primary Product using this most advanced and sophisticated method with proven accuracy is shown in Table 5. Hence, the Panel considered these values as more reliable than those presented in Table 4.

Table 5: Quantification of selected volatile constituents of the Primary Product

CAS no.	Chemical name	Batch no. (g/kg)					Average (g/kg)	Average (wt%)
		10300221	10300229	10300233(#)	10300236(#)	10300239		
110-13-4	2,5-hexanedione	0.1	0.11	0.13	0.1	0.1	0.1 ± 0.01	0.01 ± 0.00
123-31-9	1,4-benzenediol	1.5	1.6	1.6	1.6	1.8	1.6 ± 0.1	0.16 ± 0.00
120-80-9	Benzene-1,2-diol (1,2-benzenediol)	11	12	12	11	13	11.8 ± 0.8	1.18 ± 0.08
488-17-5	1,2-benzenediol, 3-methyl-	3.0	3.1	3.5	3.4	3.5	3.3 ± 0.2	0.33 ± 0.02
452-86-8	1,2-benzenediol, 4-methyl-	2.8	3.0	3.3	3.1	3.4	3.1 ± 0.2	0.31 ± 0.02
1003-29-8	Pyrrole-2-carbaldehyde (1*H*-pyrrole-2-carboxaldehyde)	0.03	0.04	0.05	0.05	0.06	0.04 ± 0.01	0.004 ± 0.00
497-23-4	Furan-2(5*H*)-one (2(5*H*)furanone)	4.1	3.4	4.2	3.7	3.8	3.8 ± 0.3	0.38 ± 0.03

Each batch was subjected to two replicate measurements, and results are expressed as g/kg.
wt: weight.
(#): Batches tested in the toxicological studies.

According to the information provided by the applicant (Documentation submitted to EFSA No. 2; Table 1), the total volatile fraction of Smoke Concentrate 809045 accounted for 44.1 wt% of the Primary Product. On average the proportion of identified and quantified volatiles amounted to approximately 83 wt% of the total volatile fraction; thus, 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).

Following an additional data request from EFSA, the applicant commented on the fact 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 Smoke Concentrate 809045 (EFSA CEF Panel, 2009). The applicant emphasised that this is not due to changes in the manufacturing process and/or changes in composition of the Primary Product. Explanations provided by the applicant were: inter-laboratory variation, the use of state-of-the-art equipment, method improvements and mostly, the prevention of artefacts produced during the analytical investigation, e.g. by the implementation of an injection system with an injector temperature limited to 125 °C. The newly developed method takes into account the recommendation of the EFSA Guidance for the preparation of applications on smoke flavouring Primary Products (EFSA FAF Panel, 2021) (Documentation provided to EFSA no. 2). The Panel acknowledges this explanation.

3.1.2.4.3. Non-volatile fraction

The applicant provided information on the non-volatile fraction for two batches of the Primary Product (10300233# and 10300236#). The non-volatile fraction of the Primary Product obtained after removal of the volatile constituents via vacuum distillation amounted to approx. 47 wt% (Documentation provided to EFSA No. 2). This non-volatile fraction of the Primary Product was separated into a water-insoluble precipitate (solid I) and an aqueous filtrate from which a water-soluble residue (solid II) was obtained by freeze-drying. Based on the analyses of two batches of the Primary Product, the water-insoluble precipitate (solid I) amounted to approximately 19 wt% and the water-soluble part (solid II) to approximately 28 wt%.

This isolated water-insoluble precipitate (solid I) was identified as 'pyrolytic lignin', i.e. a higher molecular weight material derived from lignin. Regarding this identification, the applicant referred to the data reported in the previously submitted technical dossier for Smoke Concentrate 809045 (2005). In the previous application, structural elucidation had been mainly based on pyrolysis-GC–MS analysis. Approximately 92% of the total peak area detected upon pyrolysis-GC–MS (pyrolysis temperature 475 °C) of the isolated water-insoluble precipitate was assigned to phenolic lignin-derived compounds.

In the present application, the applicant submitted additional data to gain more detailed information on the non-volatile part of the Primary Product. The performed investigations encompassed different analytical techniques, i.e. infrared spectroscopy, thermogravimetric analysis, size exclusion chromatography, LC–MS, pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS) and nuclear magnetic resonance. The applicant applied these techniques to both solid I and solid II. The Panel considered the data obtained by these techniques to support the division of the non-volatile fraction of the Primary Product into two subfractions. The obtained results confirmed the identification of solid I (approximately 19 wt%) as lignin-derived material; the application of LC–MS analysis did not reveal individual constituents, supporting its polymeric nature.

For the water-soluble solid II (approximately 28 wt%), the application of these techniques supported that this part consists of products expected from the degradation of cellulose and hemi-cellulose. The application of LC–MS resulted in the detection of individual constituents of which, however, only one could be identified.

3.1.2.4.4. Unidentified fraction

The unidentified fraction of the Primary Product amounts to approximately 36 wt% and comprises the unidentified volatile fraction and the unidentified non-volatile fraction; for the individual values see Table 1.

3.1.2.4.5. Overall Composition of the Primary Product

Based on the chemical analyses performed on two production batches of the Primary Product (Table 1), the overall composition of Smoke Concentrate 809045 (wt% of Primary Product) is shown in Figure 1, whereas the composition (wt%) of the solvent-free fraction is shown in Figure 2.

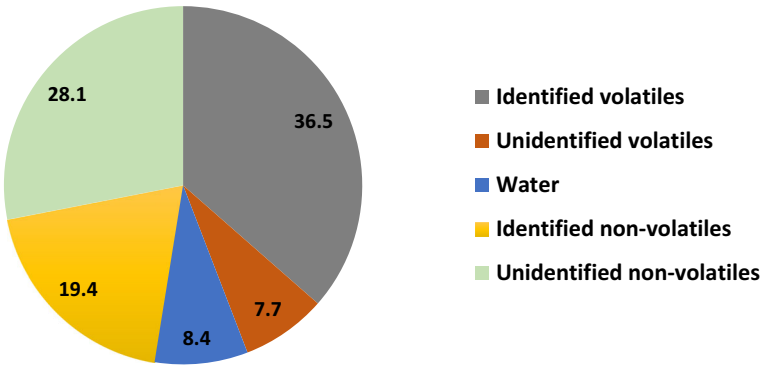

Figure 1: Overall composition of Smoke Concentrate 809045 (wt% of Primary Product)

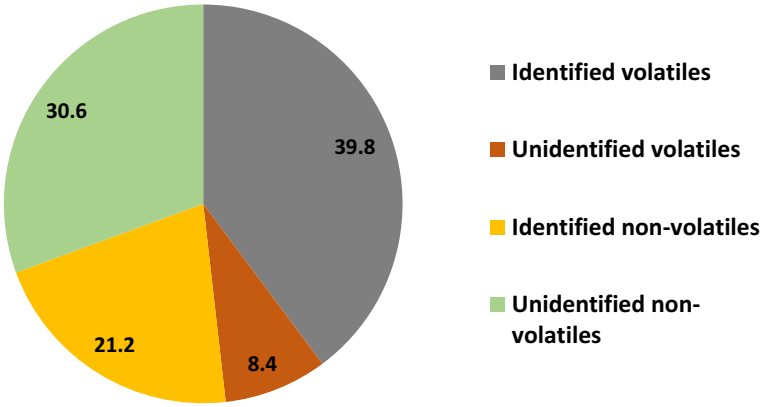

Figure 2: Composition (wt%) of the solvent-free fraction of Smoke Concentrate 809045

Regarding the identified and quantified proportion of the volatile fraction, the applied methods comply with the legal requirement that at least 80 wt% of the volatile fraction shall be identified and quantified (Regulation (EC) No 627/2006). The Panel noted that for the investigated batches of the Primary Product, the identified and quantified proportion of the solvent-free fraction is on average 61.0 wt%; thus, the applied methods meet the legal quality criterion that at least 50% by mass (wt%) of the solvent-free fraction shall be identified and quantified (Regulation (EC) No 627/2006).

3.1.2.5. Polycyclic aromatic hydrocarbons (PAHs)

Analytical data on the content of 25 PAHs were provided for 12 batches of the Primary Product. The levels reported for the 16 PAHs laid out in Regulation (EC) No 627/2006 and in EFSA scientific guidance (EFSA FAF Panel, 2021) are consistently below the minimum required limits of quantification. The performance of the analytical method is better than required by Regulation (EC) No 627/2006. For the remaining nine PAHs (indicated with an asterisk in Table 6) there are no legal requirements regarding the performance criteria of the applied analytical methods. The Panel considered that these nine additional PAHs are covered by PAH4 (see Section 3.3.3.2), and therefore there was no need for assessing them separately.

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 6: Concentrations of PAHs in the Primary Product, average from 12 batches (for batch numbers see Table 2) (Documentation provided to EFSA No. 1)

PAH	Conc. range (μg/kg)	Average (μg/kg)	SD
Phenanthrene(*)	53.9–205	123	37.9
Anthracene(*)	< 0.5(b)–26.4	17.6	< 6.6
Fluoranthene(*)	7.2–34.7	18.0	8.0
Pyrene(*)	1.0–38.7	19.6	10.4

PAH	Conc. range (µg/kg)	Average (µg/kg)	SD
Benzo[a]anthracene[(a)]	1.0–2.7	1.6	0.7
Chrysene[(a)]	0.6–2.2	1.2	0.5
Benzo[b]fluoranthene[(a)]	< 0.5[(b)]–0.8	< 0.6	< 0.1
Benzo[k]fluoranthene	< 0.5[(b)]		–
Benzo[j]fluoranthene	< 0.5[(b)]		–
Benzo[a]pyrene[(a)]	< 0.5[(b)]–0.8	< 0.5	< 0.1
Indeno[1,2,3-cd]pyrene	< 0.5[(b)]	–	–
Dibenzo[a,h]pyrene	< 1[(b)]	–	–
Benzo[g,h,i]perylene	< 1[(b)]	–	–
Dibenzo[a,l]pyrene	< 1[(b)]	–	–
Dibenzo[a,i]pyrene	< 1[(b)]	–	–
Dibenzo[a,h]anthracene	< 0.5[(b)]	–	–
Dibenzo[a,e]pyrene	< 1[(b)]	–	–
Cyclopenta[c,d]pyrene	< 1[(b)]	–	–
5-methylchrysene	< 1[(b)]	–	–
Benzo[c]fluorene	1.9–7.9	3.7	1.7
Benzo[e]pyrene[(*)]	< 1[(b)]	–	–
Perylene[(*)]	< 1[(b)]	–	–
Anthanthrene[(*)]	< 1[(b)]	–	–
Coronene[(*)]	< 1[(b)]	–	–
Benzo[b]naphtho(2,1-d)thiophene[(*)]	< 1[(b)]	–	–
PAH4	< 2.7–6.2[(c)]	< 3.9	0.9[(d)]

PAH: polycyclic aromatic hydrocarbon; SD: standard deviation.
(a): PAHs printed in bold are included in the calculation of 'PAH4', which is used for the evaluation of the exposure to these contaminants (see Section 3.3.3.2).
(b): Value below the corresponding Limit of Quantification (LOQ).
(c): Values for range of PAH4 represent the PAH4 values for the individual batches.
(d): Value calculated as the square root of the summed variances.
(*): PAH not included in the requirements in Regulation (EC) No 627/2006 and in EFSA scientific guidance (EFSA FAF Panel, 2021).

3.1.2.6. Batch-to-batch variability

The batch-to-batch variability of the Primary Product was investigated in 12 production batches (Table 7). The variability was assessed for 31 individual components of the volatile fraction. Depending on the type of constituent, different validated analytical approaches based on GC/MS–MS, LC/MS, IC and GC/MS were applied (Documentation provided to EFSA No. 1). In addition, the applicant provided data on the batch-to-batch variability as regards the contents of total acids, carbonyls and phenols by determining the respective sum parameters (Table 2). The Panel considered the variability of both the individual volatile constituents and the chemical classes in the investigated batches, with production dates spanning a period of 10 months, as acceptable. Information on the criteria underlying the selection of these batches was not provided.

Table 7: Batch-to-batch variability of the Primary Product

CAS no.	Chemical name	Batch no. (production date)												Average (wt%)	SD	RSD (%)
		221 (08-10-2020)	223 (09-11-2020)	229 (05-02-2021)	231 (10-03-2021)	232 (31-03-2021)	233 (20-04-2021)	234 (06-05-2021)	235 (31-05-2021)	236 (14-06-2021)	237 (30-06-2021)	238 (22-07-2021)	239 (06-08-2021)			
64-19-7	Acetic acid[c]	7.49	7.64	7.26	6.64	6.78	7.04	6.69	6.85	7.25	6.37	6.95	7.88	7.1	0.4	6.3
498-07-7	β-D-glucopyranose, 1,6-anhydro-[b]	5.62	4.85	5.76	4.71	5.11	5.17	4.66	5	4.77	5.76	4.14	5.62	5.1	0.5	10.0
91-10-1	Phenol, 2,6-dimethoxy-[a]	2.29	2.14	1.76	1.94	2.12	1.97	2.06	1.99	1.98	2.03	1.99	1.91	2.0	0.1	6.5
6638-05-7	Phenol, 2,6-dimethoxy-4-methyl-[a]	1.41	1.34	1.13	1.24	1.4	1.31	1.35	1.33	1.34	1.36	1.31	1.18	1.3	0.1	6.5
120-80-9	1,2-benzenediol[b]	1.11	1.26	1.19	1.27	1.21	1.18	1.22	1.16	1.25	1.21	1.19	1.45	1.2	0.1	6.8
64-18-6	Formic acid[c]	0.88	1.06	0.98	0.84	0.8	0.89	0.74	0.9	0.89	0.7	0.82	0.99	0.9	0.1	11.9
10374-51-3	2(3*H*)-furanone, dihydro-5-(hydroxymethyl)[b]	0.7	0.7	0.7	0.8	0.8	0.8	0.9	1.0	0.9	0.9	0.9	1.1	0.8	0.1	13.9
934-00-9	1,2-benzenediol, 3-methoxy-[b]	0.57	0.71	0.64	0.7	0.74	0.78	0.76	0.79	0.79	0.8	0.83	0.79	0.7	0.1	10.2
765-70-8	1,2-cyclopentanedione, 3-methyl-[a]	0.76	0.72	0.63	0.73	0.74	0.75	0.75	0.74	0.7	0.72	0.67	0.67	0.72	0.04	5.58
14059-92-8	Phenol, 4-ethyl-2,6-dimethoxy-[a]	0.71	0.72	0.6	0.63	0.76	0.69	0.71	0.7	0.7	0.7	0.69	0.64	0.69	0.04	6.36
116-09-6	2-propanone, 1-hydroxy-[d]	0.81	0.6	0.56	0.5	0.63	0.69	0.61	0.6	0.59	–	0.56	–	0.62	0.08	13.7
497-23-4	2(5*H*)-furanone[b]	0.52	0.55	0.48	0.58	0.58	0.64	0.61	0.65	0.63	0.61	0.67	0.57	0.59	0.06	9.42
19037-58-2	2-propanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-[a]	0.59	0.57	0.51	0.6	0.65	0.58	0.61	0.58	0.6	0.59	0.59	0.53	0.58	0.04	6.17
16874-33-2	2-furancarboxylic acid, tetrahydro-[a]	< 0.46	0.55	< 0.46	0.48	0.54	0.49	0.53	0.53	0.54	0.59	0.5	0.58	<0.53	<0.04	6.74
141-46-8	Acetaldehyde, hydroxy-[d]	0.72	0.28	0.23	0.5	0.61	0.66	0.54	0.53	0.59	–	0.59	–	0.53	0.16	29.8
452-86-8	1,2-benzenediol, 4-methyl-[b]	0.36	0.41	0.38	0.38	0.38	0.38	0.39	0.4	0.4	0.37	0.38	0.51	0.40	0.04	9.8
488-17-5	1,2-benzenediol, 3-methyl-[b]	0.37	0.4	0.38	0.39	0.39	0.38	0.36	0.4	0.4	0.37	0.38	0.49	0.39	0.03	8.5
20675-95-0	Phenol, 2,6-dimethoxy-4-(1E)-1-propen-1-yl-[d]	0.42	0.39	0.36	0.37	0.42	0.37	0.39	0.38	0.39	–	0.42	–	0.39	0.02	5.7

CAS no.	Chemical name	Batch no. (production date)												Average (wt%)	SD	RSD (%)
		221 (08-10-2020)	223 (09-11-2020)	229 (05-02-2021)	231 (10-03-2021)	232 (31-03-2021)	233 (20-04-2021)	234 (06-05-2021)	235 (31-05-2021)	236 (14-06-2021)	237 (30-06-2021)	238 (22-07-2021)	239 (06-08-2021)			
118-71-8	4*H*-pyran-4-one, 3-hydroxy-2-methyl-(b)	0.29	0.33	0.3	0.31	0.32	0.33	0.34	0.35	0.37	0.37	0.35	0.26	0.33	0.03	10.1
2478-38-8	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-(a)	0.29	0.29	0.25	0.28	0.31	0.29	0.29	0.29	0.29	0.28	0.28	0.24	0.28	0.02	6.7
6627-88-9	Phenol, 2,6-dimethoxy-4-(2-propen-1-yl)-(a)	0.24	0.26	0.22	0.24	0.29	0.27	0.26	0.26	0.25	0.24	0.26	0.23	0.25	0.02	7.5
5058-01-5	2*H*-pyran-2-one, tetrahydro-3-hydroxy-(d)	0.3	0.27	0.22	0.26	0.26	0.25	0.22	0.21	0.23	–	0.24	–	0.25	0.03	11.2
96-48-0	2(3*H*)-furanone, dihydro-(b)	0.21	0.25	0.22	0.23	0.23	0.26	0.25	0.24	0.25	0.25	0.27	0.25	0.24	0.02	7.1
107-21-1	1,2-ethanediol(d)	0.21	0.21	0.23	0.24	0.24	0.23	0.25	0.25	0.23	–	0.26	–	0.24	0.02	7.0
2503-46-0	2-propanone, 1-(4-hydroxy-3-methoxyphenyl)-(a)	0.23	0.22	0.2	0.22	0.25	0.22	0.23	0.22	0.23	0.23	0.22	0.21	0.22	0.01	5.5
123-31-9	1,4-benzenediol(a)	0.2	0.2	0.17	0.18	0.19	0.18	0.19	0.17	0.17	0.18	0.18	0.17	0.18	0.01	6.1
67-47-0	2-furancarboxaldehyde, 5-(hydroxymethyl)-(b)	0.13	0.09	0.1	0.15	0.14	0.17	0.16	0.19	0.21	0.2	0.21	0.38	0.18	0.08	42.4
26624-13-5	Phenol, 2,6-dimethoxy-4-(1*Z*)-1-propen-1-yl-(d)	0.16	0.17	0.18	0.17	0.17	0.18	0.17	0.18	0.17	–	0.16	–	0.17	0.01	4.3
93-51-6	Phenol, 2-Methoxy-4-methyl-(a)	0.16	0.13	0.14	0.13	0.16	0.17	0.19	0.14	0.14	0.12	0.16	0.13	0.15	0.02	13.9
90-05-1	Phenol, 2-methoxy-(a)	0.15	0.13	0.15	0.12	0.14	0.15	0.17	0.12	0.12	0.1	0.14	0.13	0.14	0.02	13.9
5650-43-1	1-propanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-(a)	0.12	0.12	0.11	0.12	0.13	0.12	0.13	0.12	0.12	0.12	0.12	0.1	0.12	0.01	6.7

wt: weight; SD: standard deviation; RSD: relative standard deviation.
(a): Quantifications based on GC–MS/MS using external calibration.
(b): Quantifications based on LC–MS using external calibration.
(c): Quantifications based IC using external calibration.
(d): Quantifications based on GC/MS using external calibration.

3.1.2.7. Solubility and particle size

Water solubility and particle size of Smoke Concentrate 809045 were not determined by the applicant. However, the Panel considered that Smoke Concentrate 809045 is an aqueous viscous liquid. It is subjected to different purification steps during the manufacturing process (Section 3.1.1.2). In particular, the Primary Product is obtained by absorption of smoke condensate in water/ethanol. This aqueous solution is subsequently treated with charcoal and subjected to filtration and concentration. Taking this sequence of production steps into account, the Panel considered it unlikely that small particles including nanoparticles are present in the final Primary Product.

3.1.3. Specifications

The applicant provided the required product specification data and reported that the Primary Product is manufactured within its proposed specification (Documentation provided to EFSA No. 1, 2 and 5). Information on the parameters considered to be relevant for the specifications has been compiled by the Panel in Table 8.

Table 8: Relevant information for specifications of the Primary Product

	Specifications for Smoke Concentrate 809045 as proposed by the applicant	**Specifications as reported in EFSA CEF Panel (2009)**	**Specifications as laid down in Regulation (EU) No 1321/2013**
Description	Smoke flavouring Primary Product obtained from beech (*Fagus sylvatica*)		
Source material:			
• Wood	100% beech (*Fagus sylvatica*)		Beech (*Fagus sylvatica*)
Identity parameters:			
• Physico-chemical parameters			
– pH	2.0–3.2		2–3
– Density	1,225–1,295 g/L		
– Refraction index	1.48–1.56	1.48–1.56	
– Staining index	n.d.		
– Extinction (400 nm, 0.5% methanol, 1 cm)	1.8–2.7		
– Flash point	> 100°C	> 100°C	
Chemical composition:			
• Chemical classes:			
– Acids	8–15 wt%(as acetic acid)		8–15%(as acetic acid)
– Carbonyls	10–20 wt% (as 2-butanone)		10–20%
– Phenols	0.20–0.65 wt% (as 2,6-dimethoxyphenol)		0.2–0.6%
– Water	5–15 wt%	5–15 wt%	5–15%
20 principal constituents of the volatile fraction	See Table 4		
Purity:			
• Benzo[a]pyrene	< 10 μg/kg	< 10 μg/kg	
• Benzo[a]anthracene	< 20 μg/kg	< 20 μg/kg	
• Sum of 4 PAHs(1)	≤ 40 μg/kg		
• Toxic elements			
– Lead	< 5 mg/kg	< 10 mg/kg	< 5 mg/kg
– Arsenic	< 3 mg/kg	< 1 mg/kg	< 3 mg/kg
– Cadmium	< 1 mg/kg	< 1 mg/kg	< 1 mg/kg
– Mercury	< 1 mg/kg	< 1 mg/kg	< 1 mg/kg

n.d.: not determined.
(1): The four PAHs are: benzo[a]pyrene, chrysene, benzo[a]anthracene and benzo[b]fluoranthene.

The Panel noted that the analytical data for the batches analysed indicated that actual concentrations of toxic elements and PAHs, given in Tables 3 and 6, are substantially lower than the currently proposed limits (Table 8), being the same as the limits laid down in the respective Regulations (i.e. Regulation (EU) No 1321/2013 for toxic elements and Regulation (EC) No 2065/2003 for benzo[a]pyrene and benzo[a]anthracene).

The parameter of the Staining index has not been provided by the applicant. However, the Panel considered that this was not of relevance for the safety assessment.

The Panel considered that the proposed extension in the specifications of the range of pH and phenols (see Table 8) could be justified by the newly provided compositional data (see Section 3.1.2.4.1).

3.1.4. Stability and fate in food

Stability tests were performed on 12 batches of the Primary Products (for batch numbers see Table 2). The tests were performed at ambient temperature by monitoring the concentrations of 31 selected compounds, representative of the chemical classes of the volatile fraction of the Primary Product, by GC–MS, GC–MS/MS, LC–MS and IC analyses, respectively. The applicant submitted a technical report containing the validation parameters of the methods employed (Documentation provided to EFSA No. 1). The selected components were measured every 3 months for 9 months, starting in August/September 2021 finishing in April/May 2022. Based on the observed relative standard deviations for the individual constituents (on average below 10%), their concentrations were sufficiently stable under the tested conditions. According to the applicant, some batches had already been stored up to 10 months under ambient conditions before commencing the analyses; thus, the testing of the storage stability of the Primary Product could be estimated to cover a period of at least 18 months. On the basis of these data, the Panel considered the stability of the Primary Product upon storage under the intended conditions not to be of concern. No data on the stability of the Primary Product in commercial formulations or in the proposed food categories were provided.

3.2. Proposed uses and use levels

The applicant applied for a renewal of authorisation of the Primary Product Smoke Concentrate 809045 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).

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)]
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	Only fresh raw sausages	200	100
08.3	Meat products	Only Luncheon spiced ham-type tinned meat, meat specialties, cured seasoned pork meat, cooked cured (or seasoned) meat (except ovine), preserved or partly preserved sausages	200	100
12.2.2	Seasonings and condiments	Only smoke flavoured salt	1,000	600
12.5	Soups and broths	Only meat soups, vegetable soups, legume soups and meat stock cubes or granulate	300	100
12.6	Sauces	Only tomato sauces, barbecue sauces, aioli or garlic sauce	1,000	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)]
12.7	Salads and savoury based sandwich spreads	Only *Feinkostsalat*	1,000	600
12.9	Protein products, excluding products covered in category 1.8		1,000	600
15.1	Potato-, cereal-, flour- or starch-based snacks		750	400
15.2	Processed nuts	Only processed dried nuts	750	400

(a): Use levels are provided for the foods as consumed.

The applicant also proposed maximum and expected typical use levels for the Primary Product in composite dishes so to cover foods which could contain 'meat, fish and sauce ingredients containing smoke flavourings' (e.g. meat based dishes, legumes based dishes, sandwich and sandwich-like dishes, pizza and pizza-like dishes, finger food, pasta based dishes (cooked)). These foods and their proposed maximum and expected typical use levels are not listed in Table 8 because, in line with article 18(1) (a) and (c) of Regulation N°1333/2008, authorisations in prepared or composite dishes are covered by the authorisations in the relevant respective food categories. The composite dishes as mentioned by the applicant were considered in the exposure assessment, as described below.

3.3. Exposure

3.3.1. Food consumption data used for the exposure assessment

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

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

Table 10: Population groups and countries considered for the exposure estimates of the Primary Product obtained 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, the Netherlands, Portugal, Slovenia, Spain
Children[(c)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, the Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia,

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

Population	Age range	Countries with food consumption surveys covering more than 1 day
		the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[(c)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

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

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

3.3.2. Exposure assessment of the Primary Product

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

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

3.3.2.1. Exposure assessment using FAIM

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

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 8. This tool does not allow to include or exclude specific foods from the exposure assessment.

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

The composite foods for which the applicant provided maximum and expected typical use levels were allocated to the relevant food categories based on their main ingredient at the use levels provided for these food categories. See Annex A1 for the food categories and use levels considered in FAIM.

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

	Infants (12 weeks–11 months) (n = 11/9)	Toddlers (12–35 months) (n = 15/13)	Children (3–9 years) (n = 19/19)	Adolescents (10–17 years) (n = 21/20)	Adults (18–64 years) (n = 22/22)	The elderly (≥ 65 years) (n = 22/21)
Proposed maximum use level exposure assessment scenario						
Mean	0.02–4.0	0.4–8.0	0.3–8.6	0.2–4.3	0.2–2.3	0.1–1.5
95th percentile	0.03–9.8	1.2–40.6	1.0–35.7	0.7–18.5	0.5–12.3	0.4–8.8
Expected typical use level exposure assessment scenario						
Mean	0.01–1.5	0.2–2.9	0.2–3.2	0.1–1.7	0.1–1.1	0.1–0.8
95th percentile	0.01–3.5	0.6–14.2	0.5–12.1	0.4–6.3	0.3–4.2	0.2–3.0

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

Exposure estimates using FAIM

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

At the proposed maximum use levels, the mean exposure to the Primary Product from its use as a smoke flavouring ranged from 0.02 mg/kg bw per day in infants to 8.6 mg/kg bw per day in children. The 95th percentile of exposure to the Primary Product ranged from 0.03 mg/kg bw per day in infants to 40.6 mg/kg bw per day in toddlers.

At the expected typical use levels, the mean exposure ranged from 0.01 mg/kg bw per day in infants to 3.2 mg/kg bw per day in children, and the 95th percentile of exposure from 0.01 mg/kg bw per day in infants to 14.2 mg/kg bw per day in toddlers.

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

3.3.2.2. Exposure assessment using DietEx

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

An examination of the FoodEx2 codes showed that some FoodEx2 codes belonging to a particular food category were missing based on the restrictions/exceptions provided by the applicant. Also, the

applicant had selected FoodEx2 codes that were not available in DietEx. In the first case, for instance, the applicant did not include all FoodEx2 codes covering FC 15.2 Processed nuts, such as the FoodEx2 codes for tree nuts. Such omissions in the Foodex2 codes selection were corrected by EFSA.

The corrections applied by EFSA to the food categories considered for the exposure assessment using DietEx are:

- It was not possible to distinguish smoke flavoured salt and therefore, all FoodEx2 codes related to salt (FC 12.1.1) were considered.
- Regarding vegetable/legume soups, all available vegetable/legume soups (belonging to FC 12.5 Soup and broths) within FoodEx2 nomenclature were taken into account. If dry soups were considered, the use levels were multiplied by a factor of 10 to account for water content.
- For the FC 12.6 sauces, FoodEx2 code aioli or garlic sauce was also considered.
- Concerning FC 12.7 Salads and savoury based sandwich spreads with restriction only *Feinkostsalat* as proposed by the applicant, FoodEx2 codes of prepared salads were taken into account.
- For FC 12.9 Protein products excluding products covered in category 1.8, all FoodEx2 codes related to meat imitates were considered.
- FC 15.1 Potato-, cereal-, flour- or starch-based snacks as proposed by the applicant was considered by taking into account all FoodEx2 codes related to fried or extruded cereal, seed or root-based products.
- FC 15.2 Processed nuts: all FoodEx2 codes That belong to this food category were considered.
- The applicant also proposed to use the Primary Product in composite dishes restricted to 'Only foods containing meat, fish and sauces ingredients', therefore all composite dishes containing meat and/or fish and/or sauce as ingredient were considered in this assessment. When a FoodEx2 code for a dish with such ingredient(s) was not available in DietEx, its parent code was considered if this did not result in an unrealistically large overestimation of the exposure, based on expert judgement (e.g. 'Omelette with Bacon' is not available in DietEx and considering its parent food group 'Egg-based dishes' would grossly overestimate the dietary exposure). The use levels proposed by the applicant were used in the assessment and adjusted by a factor (see Annex A4), based on the percentage of meat and sauce in the dishes as proposed by the applicant.

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

	Infants (0 weeks–11 months)[a] n = 12/11	**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)[b] n = 33/29**
Proposed maximum use level exposure assessment scenario						
Mean	0.1–0.8	0.4–1.1	0.3–1.2	0.1–1.5	0.1–1.0	0.1–0.8
95th percentile	0.2–1.9	1.1–5.2	0.9–4.3	0.5–4.9	0.4–3.7	0.2–3.0
Expected typical use level exposure assessment scenario						
Mean	0.03–0.5	0.2–0.7	0.1–0.6	0.1–0.9	0.1–0.6	0.03–0.5
95th percentile	0.1–1.0	0.5–3.1	0.4–2.5	0.3–2.9	0.2–2.2	0.1–1.8

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

(a): The number of surveys for infants is different compared to FAIM as the age range for this population group is different 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).

For the other food categories, the FoodEx2 codes selected by the applicant could be included in DietEx. See Annex A4 for the list of FoodEx2 codes per food category that were used in the exposure assessment using DietEx (see Section 3.3.2.1).

Exposure estimates using Dietex

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

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, adolescents, adults and the elderly to 1.5 mg/kg bw per day in adolescents. The 95th percentile of exposure to the Primary Product ranged from 0.2 mg/kg bw per day in infants and the elderly to 5.2 mg/kg bw per day in toddlers.

At the expected typical use levels, the mean exposure ranged from 0.03 mg/kg bw per day in infants and the elderly to 0.9 mg/kg bw per day in adolescents, and the 95th percentile of exposure from 0.1 mg/kg bw per day in infants and the elderly to 3.1 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. Also, considering the parent food for foods not available in DietEx, unless this would have resulted in an unrealistically large overestimation of the exposure, contributed to an overestimation of the exposure with DietEx.

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 most 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:

- Sausages which belonging to FC 8.2.
- Meat products belonging to FC 8.3.
- Tomato ketchup and related sauces belonging to FC 12.6.
- Fried or extruded cereal, seed or root-based products belonging to FC 15.1.

Considering the conservative nature of the underlying assumption that 100% of the foods within the FoodEx2 codes (with the restrictions/exceptions, Table 8) 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

Results show that exposure estimates by FAIM (Table 11) are higher than those by DietEx (Table 12). As explained above, together with other sources of standard uncertainties (described in Annex A9), the estimates obtained by both tools overestimate the expected exposure to the Primary Product if used at the proposed maximum or expected typical use levels (see Sections 3.3.2.1 and 3.3.2.2). However, this overestimation is less pronounced using DietEx, because it allows a better selection of the actual foods to which the Primary Product may be added, resulting in less conservative estimates of the exposure. The DietEx exposure estimates (see Table 12) will be used for the risk assessment of the Primary Product, 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, cadmium, lead, 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. 1.5 mg/kg bw per day for adolescents and 5.2 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 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 intra species differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 μg/kg bw per week, expressed as mercury was established (EFSA CONTAM Panel, 2012).
PAH4/340 μg/kg bw per day ($BMDL_{10}$)	Polycyclic aromatic hydrocarbons (PAHs) are considered genotoxic and carcinogenic. The reference point is based on a carcinogenicity study by Culp et al. (1998), as reported by the EFSA CONTAM Panel (2008), who concluded that PAH4 (i.e. the sum of benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene and chrysene) is a suitable indicator for the occurrence and toxicity of PAHs in food. The MOE should be at least 10,000 (EFSA CONTAM Panel, 2008).

bw: body weight; 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 margin of exposure (MOE) 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 12 samples of the Primary Product were reported (Table 3). The applicant proposed maximum limits for these toxic elements, which are the same as the limits in the current EU specifications (Table 8). The Panel noted that the actual measured levels of the toxic elements in commercial samples of the Primary Product were substantially lower than these limits.

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 reported values (for cadmium and lead) multiplied by the Panel by a factor of 5 and the LOQs (for arsenic and mercury) multiplied by the Panel by a factor of 10, 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 DietEx 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 Smoke Concentrate 809045 (mg/kg bw/day)	(i) Considering the presence of toxic elements at the current limits in the EU specifications for Smoke Concentrate 809045			
	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
1.5[(a)]	66.7–1,778	0.4	66.7	0.3
5.2[(b)]	19.2–513	1.5	19.2	0.9
	(ii) Considering the presence of toxic elements at the highest reported values (for Cd and Pb) multiplied by the Panel by a factor of 5, and the LOQs (for As and Hg) multiplied by the Panel by a factor of 10			
	MOE for As at 1 mg/kg	% of the TWI for Cd at 0.3 mg/kg	MOE for Pb at 1.95 mg/kg	% of the TWI for Hg at 0.05 mg/kg
1.5[(a)]	200–5,333	0.1	170.9	0.01
5.2[(b)]	57.7–1,539	0.4	49.3	0.05

bw: body weight; MOE: margin of exposure; TWI: tolerable weekly intake; LOQ: limit of quantification.

(a): Highest mean exposure level among the different population groups (proposed maximum use level exposure assessment scenario – adolescents, 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 (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). 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 reported values (for cadmium and lead) multiplied by a factor of 5 and the LOQs (for arsenic and mercury) multiplied by a factor of 10 (scenario (ii) in Table 14), the Panel concluded that for arsenic the lower ends of the ranges for the highest mean and 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 does not give rise to 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 for the 16 PAHs were reported by the applicant for 12 batches of the Primary Product (Table 6). The applicant also provided analytical data for nine additional PAHs (see Table 6).

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 6) 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 in the Primary Product, i.e. 6.2 μg/kg (Table 6). Based on this level, the Panel assessed the risk that would result if PAH4 were present in the Primary Product: (i) at the specifications limits for PAH4 in the Primary Product as proposed by the applicant (Table 8) and also (ii) at the maximum reported level of PAH4 in 12 batches of the Primary Product (Table 6). The outcome of the risk assessment for the two concentration scenarios and based on the highest mean and the highest 95th percentile DietEx 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 Smoke Concentrate 809045 (mg/kg bw/day)	MOE for PAH4
	(i) Considering the presence of PAH4 at the proposed specifications limits for PAH4 in Smoke Concentrate 809045 (40 μg/kg)
1.5[(a)]	5.7×10^6
5.2[(b)]	1.6×10^6
	(ii) Considering the presence of PAH4 at their maximum reported level in Smoke Concentrate 809045 (6.2 μg/kg)
1.5[(a)]	3.7×10^7
5.2[(b)]	1.1×10^7

bw: body weight; MOE: margin of exposure.

(a): Highest mean exposure level among the different population groups (proposed maximum use level exposure assessment scenario – adolescents, 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).

EFSA CONTAM Panel (2008) stated that PAH4 is a suitable indicator for the occurrence and toxicity of PHAs in food. Therefore, the Panel concluded that the safety assessment of the nine additional PAHs, for which analytical data have been provided, is implicitly covered by the risk assessment performed for PAH4.

Furthermore, the Panel noted that at the highest proposed maximum use level of the Primary Product in any of the food categories, i.e. 1,000 mg/kg food (Table 9), and the maximum reported level of PAH4 in the Primary Product, i.e. 6.2 μg/kg, the concentration of PAH4 in food would be 6.2×10^{-3} μg/kg food, which is far below the lowest maximum level (ML) of these contaminants in any of the foods listed in Regulation (EC) No 2023/915[11] (i.e. 1 μg PAH4/kg food).

[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, pp. 103–157.

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 129 identified and quantified components of Smoke Concentrate 809045 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).

Out of the 129 identified components, the applicant reported that 66 have already been evaluated by EFSA and/or JECFA/COE and were concluded by the applicant not to represent genotoxicity concern. For those components, the applicant referred to EFSA's conclusions on the genotoxic potential as set out in the respective Scientific Opinions of EFSA.

For one component, i.e. furan-2(5*H*)-one (CAS No: 497-23-4; formerly [FL-no. 10.066]), EFSA previously concluded that based on the available data, the substance is genotoxic *in vivo* (EFSA FAF Panel, 2019). Therefore, for this substance further experimental studies were performed by the applicant (see Table 16; Appendices B and D).

For the remaining 62 identified components, applicant's conclusions were based on literature search as well as an *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 toxicity prediction tool Derek Nexus (version 6.1.0),[12] applying the following models:

- Mutagenicity *in vitro* and *in vivo*
- Chromosome damage *in vitro* and *in vivo*

The Organisation for Economic Co-operation and Development (OECD) QSAR Toolbox v. 4.5[13] was also used by the applicant to complement the *in silico* analysis, applying the following profilers:

- DNA alerts for Ames, Chromosomal Aberration (CA) and Micronucleus Test (MNT) by OASIS
- Protein binding alerts for chromosomal aberration by OASIS
- DNA binding by OASIS
- DNA binding by OECD

Those components, for which potential concern for genotoxicity could not be totally dismissed by the applicant on the basis of literature and *in silico* data, were moved to the next step, including read-across considerations or experimental *in vitro* and *in vivo* genotoxicity testing.

In particular, for six individual components the applicant provided experimental data as reported in Table 16.

Results of *in vitro* and *in vivo* studies on these individual components are described in Appendix B and summarised in Appendix D.

[12] https://www.lhasalimited.org/products/derek-nexus.htm
[13] https://www.oecd.org/chemicalsafety/oecd-qsar-toolbox.htm

Table 16: List of experimental data provided for individual components of the Primary Product

CAS No.	Chemical name	Study	EFSA assessment	Reference
934-00-9	3-methoxycatechol	*In vitro* bacterial reverse mutation test	Appendices B and D	ICCR (2022a)
		In vitro MN test		ICCR (2022b)
29393-32-6	5-acetyldihydro-2(3*H*)-furanone (Soleron)	*In vitro* bacterial reverse mutation test	Appendices B and D	ICCR (2022c)
		In vitro MN test		ICCR (2022d)
108-97-4	4*H*-pyran-4-one	*In vitro* bacterial reverse mutation test	Appendices B and D	ICCR (2022e)
		In vitro MN test		ICCR (2022f)
932-66-1	1-acetyl-1-cyclohexene	*In vitro* bacterial reverse mutation test	Appendices B and D	ICCR (2022g)
		In vitro MN test		ICCR (2022h)
497-23-4	Furan-2(5*H*)-one	*In vivo* MN test in liver	Appendices B and D	LSIM (2022a)
1003-29-8	Pyrrole-2-carbaldehyde	*In vivo* combined MN and comet assay	Appendices B and D	BSRC (2023)

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).

In line with the EFSA guidance on smoke flavourings (EFSA FAF Panel, 2021), the Panel also conducted a (Q)SAR analysis for all the 129 identified and quantified components of the Primary Product using the following six profilers as available in the OECD QSAR Toolbox v. 4.5:

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

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 114 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, 1,6-heptadien-4-ol (CAS No. 2883-45-6), no genotoxicity data were available, but in absence of any structural alerts for genotoxicity, no concern for genotoxicity for this constituent was identified (see Annex B).
iii) for one substance, β-D-glucopyranose, 1,6-anhydro- (CAS No. 498-07-7), no genotoxicity data were available. Regarding the (Q)SAR analysis, a weak indication for potential genotoxicity was identified for one of the profilers, i.e. '*in vivo* mutagenicity (Micronucleus) alerts by ISS: H-acceptor-path3-H-acceptor' (see Annex B). However, no structural alerts for genotoxicity were identified by any of the other five profilers of the OECD QSAR Toolbox. Together with the consideration that the ring size (6-membered) of the oxane, resulting from the dehydration of glucose, indicates stability of the molecule, the indication for

genotoxicity of this target substance based on (Q)SAR analysis is alleviated and it is not further considered.

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

v) for the eleven components listed in Table 17 the Panel identified a 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 Appendices B and D).

For the two components for which a concern for genotoxicity has been identified as well as for the components listed in Table 17 the available genotoxicity data are described in Appendix B.

The Panel investigated if the concern for genotoxicity for furan-2(5*H*)-one and for benzene-1,2-diol and the potential concern for genotoxicity for the components listed in Table 17 could be ruled out by application of the threshold of toxicological concern (TTC) approach for DNA-reactive mutagens and/or

Table 17: List of the components of the Primary Product for which a potential concern for genotoxicity has been identified

CAS No.	Chemical name	Reference
934-00-9	3-methoxycatechol	Appendices B and D
141-46-8	hydroxyacetaldehyde	Appendix B
N/A	2-(hydroxymethyl)-5-methoxy-2-methyl-tetrahydrofuran-3-one	Appendix B
108-97-4	4H-pyran-4-one	Appendices B and D
110-13-4	2,5-hexanedione	Appendix B
Derivatives of furan-2(5*H*)-one		
591-11-7	2(5*H*)-furanone, 5-methyl-	Appendix B
22122-36-7	2(5*H*)-furanone, 3-methyl-	
26329-68-0	2(5*H*)-furanone, 5-ethyl-3-methyl-	
33488-51-6	2(5*H*)-furanone, 3,4,5-trimethyl-	
1575-46-8	2(5*H*)-furanone, 3,4-dimethyl-	
6124-79-4	2(5*H*)-furanone, 4-methyl-	

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.

For these calculations, the concentrations of the individual components in the Primary Product were used with the following priority:

i) Concentrations determined by using isotope-labelled internal standards, when available (see Table 5);

ii) Concentrations determined by the use of various approaches using external standards (see Appendix A); and

iii) Concentrations determined by the semi-quantifications based on response factors predicted by the IOFI model (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 208 above this TTC value (see Table 18) 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 eleven 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 18: Dietary exposure in μg/kg body weight (bw) per day to the thirteen 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 12)

CAS No.	Chemical name	Average content in the Primary Product (wt%)	Exposure	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)	Ratio between the highest exposure estimate and TTC
Components of concern for genotoxicity										
120-80-9	Benzene-1,2-diol (1,2-benzenediol, catechol)	1.18[(a)]	Mean	1.2–9.4	4.7–13.0	3.5–14.2	1.2–17.7	1.2–11.8	1.2–9.4	2.4544×10^4
			95th percentile	2.4–22.4	13.0–61.4	10.6–50.7	5.9–57.8	4.7–43.7	2.4–35.4	
497-23-4	Furan-2(5*H*)-one (2(5*H*)furanone)	0.38[(a)]	Mean	0.4–3.0	1.5–4.2	1.1–4.6	0.4–5.7	0.4–3.8	0.4–3.0	7.904×10^3
			95th percentile	0.8–7.2	4.2–19.8	3.4–16.3	1.9–18.6	1.5–14.1	0.8–11.4	
Components for which a potential concern for genotoxicity is identified										
934-00-9	3-methoxycatechol	0.83	Mean	0.8–6.6	3.3–9.1	2.5–10.0	0.8–12.5	0.8–8.3	0.8–6.6	1.7264×10^4
			95th percentile	1.7–15.8	9.1–43.2	7.5–35.7	4.2–40.7	3.3–30.7	1.7–24.9	
141-46-8	Hydroxyacetaldehyde	0.5	Mean	0.5–4.0	2.0–5.5	1.5–6.0	0.5–7.5	0.5–5.0	0.5–4.0	10,400
			95th percentile	1.0–9.5	5.5–26.0	4.5–21.5	2.5–24.5	2.0–18.5	1.0–15.0	
6124-79-4	2(5*H*)-furanone, 4-methyl-	0.22	Mean	0.2–1.8	0.9–2.4	0.7–2.6	20.2–3.3	0.2–2.2	0.2–1.8	4.576×10^3
			95th percentile	0.4–4.2	2.4–11.4	2.0–9.5	1.1–10.8	0.9–8.1	0.4–6.6	
22122-36-7	2(5*H*)-furanone, 3-methyl-	0.16	Mean	0.2–1.3	0.6–1.8	0.5–1.9	0.2–2.4	0.2–1.6	0.2–1.3	3.328×10^3
			95th percentile	0.3–3.0	1.8–8.3	1.4–6.9	0.8–7.8	0.6–5.9	0.3–4.8	
591-11-7	2(5*H*)-furanone, 5-methyl-	0.10	Mean	0.1–0.8	0.4–1.1	0.3–1.2	0.1–1.5	0.1–1.0	0.1–0.8	2.080×10^3
			95th percentile	0.2–1.9	1.1–5.2	0.9–4.3	0.5–4.9	0.4–3.7	0.2–3.0	
N/A	2-(hydroxymethyl)-5-methoxy-2-methyl-tetrahydrofuran-3-one	0.09	Mean	0.1–0.7	0.4–1.0	0.3–1.1	0.1–1.4	0.1–0.9	0.1–0.7	1.872×10^3
			95th percentile	0.2–1.7	1.0–4.7	0.8–3.9	0.5–4.4	0.4–3.3	0.2–2.7	
108-97-4	4H-pyran-4-one	0.06	Mean	0.1–0.5	0.2–0.7	0.2–0.7	0.1–0.9	0.1–0.6	0.1–0.5	1.248×10^3
			95th percentile	0.1–1.1	0.7–3.1	0.5–2.6	0.3–2.9	0.2–2.2	0.1–1.8	
1575-46-8	2(5*H*)-furanone, 3,4-dimethyl-	0.03	Mean	0.0–0.2	0.1–0.3	0.1–0.4	0.0–0.5	0.0–0.3	0.0–0.2	624
			95th percentile	0.0–0.6	0.3–1.6	0.3–1.3	0.2–1.5	0.1–1.1	0.1–0.9	
26329-68-0	2(5*H*)-furanone, 5-ethyl-3-methyl-	0.02	Mean	0.0–0.2	0.1–0.2	0.1–0.2	0.0–0.3	0.0–0.2	0.0–0.2	416
			95th percentile	0.0–0.4	0.2–1.0	0.2–0.9	0.1–1.0	0.1–0.7	0.0–0.6	

CAS No.	Chemical name	Average content in the Primary Product (wt%)	Exposure	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)	Ratio between the highest exposure estimate and TTC
33488-51-6	2(5*H*)-furanone, 3,4,5-trimethyl-	0.02	Mean	0.0–0.2	0.1–0.2	0.1–0.2	0.0–0.3	0.0–0.2	0.0–0.2	416
			95th percentile	0.0–0.4	0.2–1.0	0.2–0.9	0.1–1.0	0.1–0.7	0.0–0.6	
110–13-4	2,5-hexanedione	0.01[(a)]	Mean	0.0–0.1	0.0–0.1	0.0–0.1	0.0–0.2	0.0–0.1	0.0–0.1	208
			95th percentile	0.0–0.2	0.1–0.5	0.1–0.4	0.1–0.5	0.0–0.4	0.0–0.3	

wt: weight; TTC: threshold of toxicological concern.
(a): Concentrations determined by using isotope-labelled internal standards (see Table 5).

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

The applicant resubmitted the genotoxicity studies on the Primary Product (whole mixture) that were already evaluated by the CEF Panel in 2009, to investigate the genotoxicity of the unidentified fraction of the Primary Product, in line with the EFSA Scientific Committee statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019): a bacterial reverse mutation test (Freiburger Labor für Mutagenitätsprüfung, 1998a), an *in vitro* mammalian cell gene mutation assay in mouse lymphoma cells (TNO, 2005), an *in vitro* mammalian chromosomal aberration test (TNO, 2004), an *in vivo* micronucleus (MN) assay in mouse bone marrow (Freiburger Labor für Mutagenitätsprüfung, 1998b) and an *in vivo* rat liver unscheduled DNA synthesis (UDS) assay (TNO, 2007).

The evaluation of these studies as described in the scientific opinion 'Safety of smoke flavour Primary Product – Smoke Concentrate 809045' (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 E.1 and E.2 (Appendix E), 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, a new *in vivo* genotoxicity study was provided, which is described in Section 3.4.2.2 and summarised in Appendix F, Table F.1.

The batch used in this newly submitted genotoxicity study (no. 10300233) fell within the reported range of batch-to-batch variability and could be considered representative (see Section 3.1.3).

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

3.4.2.1.1. Bacterial reverse mutation test (Freiburger Labor für Mutagenitätsprüfung, 1998a)

'The bacterial gene mutation assay was conducted with Salmonella Typhimurium *strains TA1535, TA1537, TA1538, TA98 and TA100 using the standard plate incorporation assay with and without metabolic activation at concentrations of 50 to 5,000 μg/plate. The experiment was repeated after a 3-day interval using the same protocol. No study was performed using a pre-incubation protocol*.

In the absence of metabolic activation, cytotoxicity was observed towards TA1537 and TA1538 at 1,500 μg/plate and towards the other strains at 5,000 μg/plate. With metabolic activation, cytotoxicity was observed towards strains TA1537, TA1538 and TA98 at 5,000 μg/plate. In the first experiment, Smoke Concentrate 809045 induced a significant concentration-dependent increase in mutation frequency in TA100, of up to 1.7 fold both with and without metabolic activation. These results were not reproduced in the second study in which the maximum mutation frequency was not increased in the presence of metabolic activation and increased only 1.3-fold in the absence of metabolic activation with no concentration dependence. Although the authors concluded from the first study that Smoke Concentrate 809045 was mutagenic to S. Typhimurium *TA100 in the presence and absence of a metabolising system, the Panel concluded that overall these results were equivocal'* (EFSA CEF Panel, 2009).

The FAF Panel agreed with the evaluation of the CEF Panel (EFSA CEF Panel, 2009). Moreover, the Panel noted that the battery of bacterial strains used was incomplete as *S*. Typhimurium TA102 or *Escherichia coli* WP2 uvrA were not included. The Panel considered the study as reliable with restrictions, due to the incomplete battery of bacterial strains used. Therefore, overall, the study is considered inconclusive, and accordingly the relevance of the study result is low.

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

'In the test for gene mutations in eukaryotic cells, mouse lymphoma L5178Y cells were incubated with Smoke Concentrate 809045 for 4 and 24 hours in the absence and presence of metabolic activation. Cytotoxicity was observed at concentrations of 26 μg/mL in the absence of S9-mix and 49 μg/mL in the presence of S9. Relatively more small than large colonies were formed in the presence and absence of S9 after 4 h which was considered to be indicative of clastogenic potential. Both in the absence and presence of S9-mix, under all conditions, positive responses were observed at dose levels causing less than 90% cell toxicity. The Panel concluded that Smoke Concentrate 809045 is mutagenic at the TK locus in L5178Y cells' (EFSA CEF Panel, 2009).

The FAF Panel confirmed the conclusion of the CEF Panel (EFSA CEF Panel, 2009). This study is positive also when applying the global evaluation factor as an additional criterion to evaluate the results, as recommended in the current OECD TG 490 (OECD, 2016a). The Panel considered the study to be reliable without restrictions and its result of high relevance.

3.4.2.1.3. In vitro *mammalian chromosomal aberration test (TNO, 2004)*

'A test for clastogenicity (chromosomal aberrations) in Chinese Hamster Ovary (CHO) cells in vitro was conducted at concentrations of 25, 50 and 125 μg/mL in the absence of metabolic activation and 100, 125 and 250 μg/mL in the presence of S9-mix; dose levels were selected based on mitotic index. In a repeat experiment on a separate occasion, concentrations of 50, 75 and 100 μg/mL were used without metabolic activation and 150, 200 and 300 μg/mL in the presence of S9-mix. In the second study, the protocol used both pulse treatment (4 h treatment harvested after 18 h) and continuous treatment for 18 h. In the first test, a statistically significant increase in aberrant cells was observed at the highest concentration tested without (but not with) metabolic activation. In the second experiment, an increase in aberrant cells was seen at the highest concentration tested in the pulse assay both in the absence and presence of metabolic activation and at the two highest concentrations in the continuous assay without metabolic activation. The Panel concluded that Smoke Concentrate 809045 was clastogenic to CHO cells' (EFSA CEF Panel, 2009).

The FAF Panel agreed that the Primary Product showed evidence of clastogenic activity in this test. 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. Therefore, the Panel considered that the study results are of limited relevance.

3.4.2.1.4. In vivo *bone marrow mouse micronucleus test (Freiburger Labor für Mutagenitätsprüfung, 1998b)*

'In an in vivo mouse micronucleus assay, Smoke Concentrate 809045 was administered orally to groups of five male and five female NMRI mice at a dose level of 2,000 mg/kg bw. Bone marrow smears were examined 24, 48 and 72 h after treatment. The test material did not induce a significant increase in micronuclei; in some animals, the PCE:NCE ratio was decreased after 72 h' (EFSA CEF Panel, 2009).

The polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) ratio was decreased in males after 24 and 72 h (0.57 and 0.49 vs. 0.80 in the control group (that was analysed after 24 h only)) but not after 48 h (0.80 vs. 0.80). The ratio of 0.57 observed at 24 h is 71% of 0.80 observed in the control group. This could be considered as an indication of bone marrow toxicity and, accordingly, of bone marrow exposure. The ratio of 0.49 observed at 72 h is 61% of 0.80 observed in the control group. The observation of bone marrow toxicity at 24 and 72 h post dosing can be considered as an indication of bone marrow exposure.

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. Considering the bone marrow toxicity, it is unlikely that higher doses could be applied.

The Panel further noted that only 1,000 cells were counted for the frequency of micronucleated cells, whereas counting of at least 2,000 cells was recommended by OECD TG 474 (OECD, 1997) and meanwhile 4,000 cells as recommended by OECD TG 474 (OECD, 2016c). This is a limitation.

It should also be noted that, according to the statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019), even in the case 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.

Overall, based on the currently applicable criteria, the FAF Panel considered this study as reliable with restrictions and the negative result of limited relevance.

3.4.2.1.5. In vivo *rat liver UDS assay (TNO, 2007)*

'In an in vivo UDS assay conducted to GLP requirements, Smoke Concentrate 809045 was administered to male Wistar rats, strain CRL: [W1] WU BR rats by gavage in corn oil at a limit dose of 2,000 mg/kg bw. Group sizes were six animals in the negative control and test groups and two to three animals respectively in positive control groups receiving 2-AAF or NDMA. After 2–4 h and 12–16 h, animals were sacrificed by perfusion and hepatocytes were isolated. Hepatocytes in monolayer culture

were incubated with [methyl-3H]-thymidine and examined by autoradiography. Animals treated with NDMA or 2-AAF served as controls at the 2–4 h and 12–16 h time points, respectively.

Smoke Concentrate 809045 did not increase the mean net nuclear grain count in hepatocytes relative to untreated control rats. The positive controls induced the expected increase in UDS. It was concluded that the validity of the assay was confirmed and that Smoke Concentrate did not induce increased UDS under the conditions of the assay' (EFSA CEF Panel, 2009).

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

3.4.2.2. New genotoxicity study

The smoke flavouring Primary Product was tested in an *in vivo* gene mutation assay in transgenic mice (BSRC, 2022a).

The concentration of the tested Primary Product in the formulations was confirmed by the analysis of selected components. The applicant submitted a validated analytical method for the determination of 2(5*H*)-furanone and 4-methyl-2,6-dimethoxyphenol as typical components of the Primary Product in propylene glycol using LC–MS/MS (BSRC, 2022b).

In the study report for the *in vivo* gene mutation assay (BSRC, 2022a), the applicant reported that the stability of test article formulations (2 and 200 mg/mL) when stored in a refrigerator for 8 days or stored at room temperature for 24 h had been confirmed in a separate study that was not submitted to EFSA.

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

Smoke Concentrate 809045 (batch no. 10300233) was tested in a 14-day dose range finding (DRF) (non-GLP) study in CD2F1/Slc male mice (i.e. wild type Muta™Mouse) in order to determine the maximum tolerated dose (MTD) and dose levels for the transgenic rodent (TGR) gene mutation assay using the same rodent strain (BSRC, 2022c).

Smoke Concentrate 809045 was administered via oral gavage (propylene glycol used as vehicle) to groups of CD2F1/Slc male mice (three animals per group) corresponding to the following dose levels: 0, 300, 600 and 1,000 mg/kg bw per day. Animals were observed daily for clinical signs.

In all groups, there were no mortalities, no clinical signs of toxicity, no bw changes and no gross findings related to treatment. Histopathological examination was performed on the stomach only and no test substance-related changes were observed. Based on these results in which 1,000 mg/kg bw per day was considered the maximum dose required by OECD TG 488 (OECD, 2020a), the study authors selected doses of 250, 500 and 1,000 mg/kg bw per day for the *in vivo* gene mutation assay with Muta™Mouse (BSRC, 2022c).

In the *in vivo* gene mutation assay in Muta™Mouse (BSRC, 2022a), Smoke Concentrate 809045 (batch no. 10300233) was administered via oral gavage to three groups of male transgenic CD2-LacZ80/HazfBR mice (Muta™Mouse) (six animals per group) at dose levels of 0, 250, 500 and 1,000 mg/kg bw per day for 28 consecutive days. This study was performed according to OECD TG 488 (OECD, 2020a) and in compliance with GLP. Propylene glycol was used as the vehicle control (12 animals per group). The treatment period was followed by a 3-day manifestation period and then animals were sacrificed, and the liver, stomach and duodenum removed. N-Ethyl-N-nitrosourea (ENU) administered intraperitoneally (six animals per group) at a dose of 100 mg/kg bw per day for 2 consecutive days followed by a 10-day manifestation period was used as a concurrent positive control. Test item formulations were prepared on an approximately weekly basis, 1–2 days prior to first use. The positive control was prepared just before use.

In all groups, there were no mortalities, no clinical signs of toxicity, no differences in bw gain or food consumption and no gross findings related to treatment observed in the liver, stomach or duodenum.

Liver, duodenum and stomach samples from five animals per test item and positive control groups were processed for DNA isolation. Conversely, all 12 animals from the vehicle control were processed for DNA isolation as the laboratory's historical control database did not contain data for propylene glycol.

For each DNA sample from vehicle control or test item-treated animals, the number of plaques from a single packaging was greater than 300,000 (i.e. more than the OECD recommended minimum of 125,000 plaques). For DNA samples from positive control-treated animals, the number of plaques from 1 to 2 packagings was greater than 300,000.

Treatment with Smoke Concentrate 809045 did not significantly increase the mutation frequency at the *lacZ* gene in liver, stomach or duodenum of Muta™Mouse mice. The mutant frequency in the duodenum in the 250 mg/kg bw per day group was slightly below the lower bound 95% confidence interval, but the study authors considered that this did not affect the evaluation of the results.

The Panel concluded that Smoke Concentrate 809045 did not induce a statistically significant increase in mutation frequency in the liver, stomach or duodenum in this study. However, the Panel noted that no signs of toxicity were observed at 1,000 mg/kg bw, the highest dose tested in the dose range finding study and in the gene mutation assay, and that it would have been appropriate to investigate whether a higher dose could have been applied as maximum tolerated dose, as per EFSA recommendation.[14] Accordingly, the Panel considered the study as reliable with restrictions and the results of limited relevance.

Results of this new *in vivo* study are summarised in Appendix F.

4. Discussion

The European Commission has requested the European Food Safety Authority (EFSA) to evaluate the safety of the smoke flavouring Primary Product Smoke Concentrate 809045 (SF-003), 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 by pyrolysis of beechwood (*F. sylvatica* L.) chips. The production of the Primary Product occurs in three main stages: (i) smoke generation, (ii) condensation and absorption of smoke and (iii) further processing of purification. 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).

For two batches, which were also used for toxicological studies and which the Panel considered as representative of the Primary Product, the identified and quantified proportion of the solvent-free fraction was higher than 50 wt%, which meets the legal quality criterion that at least 50% 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 for the two batches, the Panel noted that 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).

Data provided for 12 batches of the Primary Product demonstrated that their batch-to-batch variability was sufficiently low (i.e. the observed relative standard deviations was on average below 11%), based on the analytical data for the quantified 31 volatile constituents. The Panel noted that the applicant has adequate control over the relevant steps of the production process (pyrolysis and purification) and concluded that the data provided in the selected batches are representative of the Primary Product.

Considering the different purification steps to which the Primary Product is subjected during the manufacturing process (see Sections 3.1.1.2 and 3.1.2.7), the Panel considered it unlikely that small particles including nanoparticles are present in the final Primary Product.

The applicant proposed limits for four toxic elements (arsenic, cadmium, lead and mercury), which are the same as in the current EU specifications (Table 8). The Panel noted that the actual measured levels for these elements in 12 batches of the Primary Product (Table 3) were substantially lower than these limits.

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), (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 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 reported values (for Cd and Pb) multiplied by a factor of 5 and the LOQs (for As and Hg) multiplied by a factor of 10 (scenario (ii) in Table 14), the Panel concluded that for arsenic the lower ends of the ranges for the highest mean and 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 does not give rise to concern.

The analytical procedure for the determination of the 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

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

Primary Product and the maximum reported level of the PAH4 in the Primary Product (i.e. 6.2 μg/kg), an MOE of at least 1.1×10^7 could be calculated for the exposure to PAHs, which would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions (see EFSA Scientific Committee, 2012). The Panel noted that including a limit for PAH4 in the EU specifications would take better account of the presence of other PAHs than only the two PAHs benzo[a]pyrene and benzo[a]anthracene.

Overall, the Panel considered that limits in the EU specifications for the four toxic elements and PAH4 should be established based on actual levels in the 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 nine 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 expected to be 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 0.1 mg/kg bw per day in infants, adolescents, adults and the elderly to 1.5 mg/kg bw per day in adolescents (Table 12). The 95th percentiles DietEx exposure estimates ranged from 0.2 mg/kg bw per day in infants and the elderly to 5.2 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 and the elderly to 0.9 mg/kg bw per day in adolescents; the 95th percentile DietEx exposure estimates ranged from 0.1 mg/kg bw per day in infants and the elderly to 3.1 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 129 individual components of the Primary Product, the Panel considered that:

i) for 116 individual components no concern for genotoxicity is identified (see Annex B);
ii) a concern for genotoxicity is identified for two components, i.e. furan-2(5*H*)-one and benzene-1,2-diol, which are present in the Primary Product at average concentrations of 0.38% and 1.18%, respectively;
iii) for eleven components (see Table 17) a potential concern for genotoxicity is identified, for which additional data are needed to reach a conclusion on the genotoxic potential of these substances.

The details of the genotoxicity data available on the 13 components listed in (ii) and (iii) are given and discussed in Appendix B.

Regarding the two components furan-2(5*H*)-one and benzene-1,2-diol, the available data raise a concern for genotoxicity. As described in detail in Appendix B, furan-2(5*H*)-one induced MN in liver of rats. Based on the positive results observed in this *in vivo* MN study, the applicant submitted a study to investigate the pro-oxidative potential of furan-2(5*H*)-one and an *in vitro* MultiFlow® screen with the aim of clarifying the mode of action (MOA) and to determine whether the genotoxic effect observed is threshold-mediated. The Panel considered that there is inadequate evidence to suggest that the genotoxicity of furan-2(5*H*)-one is mediated through reactive oxygen species (ROS) production. An association of ROS production with DNA strand breaks and toxicity is not evidence of a causative role. Results from the *in vitro* MultiFlow® test on TK6 cells support a clastogenic MOA for furan-2(5*H*)-one. Overall, these new studies confirm the genotoxicity of furan-2(5*H*)-one, for which the Panel already expressed a concern in FGE.217Rev2 (EFSA FAF Panel, 2019).

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(5*H*)-one and benzene-1,2-diol and the potential concern for genotoxicity for the 11 components mentioned above in (iii) could be ruled out by application of the TTC approach for DNA-reactive mutagens and/or carcinogens (EFSA Scientific

Committee, 2019). The obtained exposure estimates were compared with the TTC value of 0.0025 μg/kg bw per day for DNA-reactive mutagens and/or carcinogens. For all the 13 substances, the exposure estimates were well above this TTC value (see Table 18) 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 13 components. However, taking into account:

- the magnitude of the calculated ratios between the exposure estimates and the above mentioned TTC value (see Table 18);
- 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 was tested in an *in vitro* bacterial gene mutation test, but the battery of bacterial strains used was incomplete and the study results were considered as inconclusive and of low relevance. However, the Primary Product tested in an *in vitro* gene mutation test in mammalian cells gave clearly positive results. In the *in vivo* follow-up study, the Primary Product did not induce gene mutations in liver, stomach and duodenum of transgenic mice. However, the Panel noted that no signs of toxicity were observed at 1,000 mg/kg bw, the highest dose tested in the dose range finding study and in the gene mutation assay, and that it would have been appropriate to investigate whether a higher dose could have been applied as maximum tolerated dose, as per EFSA recommendation. Accordingly, the Panel considered the study as reliable with restrictions and the results of limited relevance.

Therefore, this study is not strong enough to alleviate the concern for the whole mixture raised by the findings of gene mutations in an *in vitro* gene mutation assay with mammalian cells.

The Primary Product showed evidence of clastogenic activity in an *in vitro* chromosomal aberration test. The testing of the Primary Product in an *in vivo* micronucleus assay in bone marrow did not show any increase in the frequency of MNPCE. The Panel noted that bone marrow toxicity (reduction in PCE/NCE ratio) at 24 h and 72 h but not at 48 h, provided evidence of bone marrow exposure. The Panel noted that only 1,000 cells were counted for the frequency of micronucleated cells. The Panel also noted that according to the EFSA Scientific Committee statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019), even in the case 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 FAF Panel considered this study as reliable with restrictions and the negative result of limited relevance.

Therefore, this study is not strong enough to alleviate the concern for the whole mixture raised by the findings of chromosomal aberrations in an *in vitro* assay.

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 (OECD, 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 to investigate genotoxic effects at the site where the exposure to the components is expected to be maximal. However, in this case, the concern for genotoxicity for the Primary Product cannot be ruled out by an additional *in vivo* Comet assay performed on the whole mixture, since the exposure estimate for the two genotoxic components furan-2(5*H*)-one and benzene-1,2-diol are both above the TTC value of 0.0025 μg/kg bw per day for DNA-reactive mutagens and/or carcinogens. In fact, as outlined in the 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 eleven 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 Smoke Concentrate 809045 (SF-003).

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-003. The Panel concluded that the compositional data provided on the Primary Product were adequate. Furthermore, the Panel concluded that the applicant has adequate control over the production process and that the Primary Product is sufficiently stable upon storage.

Considering the different purification steps during the manufacturing process, the Panel concluded that it is unlikely that small particles including nanoparticles are present in the final Primary Product and therefore the conventional risk assessment is sufficient.

The Panel identified a potential concern for genotoxicity for eleven components in the Primary Product as well as for the unidentified fraction of the mixture. More importantly, the Primary Product contains furan-2(5*H*)-one and benzene-1,2-diol, two known *in vivo* genotoxic substances via the oral route. Considering that the exposure estimates for furan-2(5*H*)-one and benzene-1,2-diol are above the TTC of 0.0025 μg/kg bw per day (or 0.15 μg/person per day) for DNA-reactive mutagens and/or carcinogens, the Panel concluded that Smoke Concentrate 809045 (SF-003) raises concern with respect to genotoxicity.

6. Documentation as provided to EFSA

1) Dossier "Application for renewal of an already authorised smoke flavouring – Smoke Concentrate 809045". Dossier number: SFL-2021–2352. June 2022. Submitted by Symrise AG.[15]
2) Additional data received on 12 December 2022, submitted by Symrise AG in response to additional data request from EFSA sent on 11 November 2022.
3) Additional data received on 7 February 2023, submitted by Symrise AG as spontaneous submission.
4) Additional data received on 9 and 10 May 2023, submitted by Symrise AG as spontaneous submission.
5) Additional data received on 31 August 2023 submitted by Symrise AG as spontaneous submission.
6) Additional data received on 22 September 2023 submitted b Symrise AG as spontaneous submission.
7) BSRC (2022a). *In Vivo* Gene Mutation Assay of 809045 Smoke Flavouring Conc. Beechwood Type in MutaMouse. BioSafety Research Center Inc., Japan. Experiment No. J864 (673–005). April 2022. Unpublished study report submitted by Symrise AGXXX.
8) BSRC (2022b). Validation of Determination Method for 2(5*H*)-Furanone and 4-Methyl-2,6-dimethoxyphenol in 809045 Smoke Flavouring Conc. Beechwood Type [Non-GLP Study]. BioSafety Research Center Inc., Japan. Experiment No. J862 (673–003). October 2021. Unpublished study report submitted by Symrise AGXXX.
9) BSRC (2022c). Dose Range-Finding Study for Transgenic Mouse Gene Mutation Assay of 809045 Smoke Flavouring Conc. Beechwood Type [Non-GLP]. BioSafety Research Center Inc., Japan. Experiment No. J863 (673–004). July 2021. Unpublished study report submitted by Symrise AGXXX.
10) BSRC (2022d). Validation of Analytical Method for Determination of Pyrrole-2-carboxaldehyde in 0.5% (w/v) Methylcellulose [Non-GLP]. Biosafety Research Centre Inc., Japan. Experiment No. K601 (820–020). July 2022. Unpublished study report submitted by Symrise AGXXX.

[15] 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-2021-2352.

11) BSRC (2022e). Stability Study of Pyrrole-2-carboxaldehyde (CAS 1003-29-8) in 0.5% (w/v) Methylcellulose. Biosafety Research Centre Inc., Japan. Experiment No. K603 (820–022). September 2022. Unpublished study report submitted by Symrise AGXXX.
12) BSRC (2022f). Validation of an Analytical Method for Determination of Pyrrole-2-carboxaldehyde (CAS 1003-29-8) and Pyrrole-2-carboxylic acid (CAS 634–97-9) in Rat Plasma [Non-GLP]. Biosafety Research Centre Inc., Japan. Experiment No. K602 (820–021). November 2022c. Unpublished study report submitted by Symrise AGXXX.
13) BSRC (2023). Comet-micronucleus Combination Study of Pyrrole-2-carboxaldehyde (CAS 1003-29-8) in Rats. Biosafety Research Centre Inc., Japan. Experiment No. K604 (820–023). January 2023. Unpublished study report submitted by Symrise AG.
14) Charles River (2023). 2(5*H*)-Furanone *In Vitro* MultiFlow® Screen in TK6 Cells. Charles River, USA. Study No. 01907001. April 2023. Unpublished Study Report submitted by Symrise AG.
15) Fraunhofer ITEM (2023). Pro-oxidative Potential of 2(5*H*)-Furanone. Fraunhofer-Institute for Toxicology and Experimental Medicine, Germany. Study No. 17N22526. May 2023. Unpublished Study Report submitted by Symrise AG.
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18) ICCR (2022a). 3-Methoxycatechol: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay. ICCR-Roßdorf GmbH, Germany. Study No. 2183205. March 2022. Unpublished study report submitted by Symrise AG.
19) ICCR (2022b). 3-Methoxycatechol: Micronucleus Test in Human Lymphocytes *In Vitro*. ICCR-Roßdorf GmbH, Germany. Study No. 2183206. May 2022. Unpublished study report submitted by Symrise AG.
20) ICCR (2022c). Soleron: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay. ICCR-Roßdorf GmbH, Germany. Study No. 2183207. January 2022. Unpublished study report submitted by Symrise AG.
21) ICCR (2022d). Soleron: Micronucleus Test in Human Lymphocytes *In Vitro*. ICCR-Roßdorf GmbH, Germany. Study No. 2183208. June 2022. Unpublished study report submitted by Symrise AG.
22) ICCR (2022e). 4H-Pyran-4-one: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay. ICCR-Roßdorf GmbH, Germany. Study No. 2183203. January 2022. Unpublished study report submitted by Symrise AG.
23) ICCR (2022f). 4H-Pyran-4-one: Micronucleus Test in Human Lymphocytes *In Vitro*. ICCR-Roßdorf GmbH, Germany. Study No. 2183204. May 2022. Unpublished study report submitted by Symrise AG.
24) ICCR (2022g). 1-Acteyl-1-cyclohexene: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay. ICCR-Roßdorf GmbH, Germany. Study No. 2183201. March 2022. Unpublished study report submitted by Symrise AG.
25) ICCR (2022h). 1-Acteyl-1-cyclohexene: Micronucleus Test in Human Lymphocytes *In Vitro*. ICCR-Roßdorf GmbH, Germany. Study No. 2183202. July 2022. Unpublished study report submitted by Symrise AG.
26) LSIM (2021). Analytical Method Validation for Determination of Furan-2(5*H*)-one in Dosing Formulations. Safety Institute Corporation, Japan. Study No. B210495. December 2021. Unpublished study report submitted by Symrise AG.
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Abbreviations

2-AAF	2-acetylaminofluorene
AVRG	average
BMDL	benchmark dose lower limit
BSRC	Bioscience Research center
bw	body weight
CA	chromosomal aberration
CAS	Chemical Abstract Service
CBPI	cytokinesis-block proliferation index
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	Panel on Food Contact Materials, Enzymes and Processing Aids
CHO	Chinese hamster ovary
CM-H2DCF	chloromethyl-2′,7′-dichloro-dihydro-fluorescein
COE	Council of Europe
CONTAM	Panel on Contaminants in the Food Chain
DCFH	2′,7′-dichloro-dihydro-fluorescein
DLS	dynamic light scattering
DMSO	dimethyl sulfoxide
DRF	dose range finding
ECHA	European Chemicals Agency
EMS	ethyl methanesulfonate
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
FSA	Food Standards Agency
GC	gas chromatography
GC–FID	gas chromatography–flame ionisation detection
GC–MS	gas chromatography–mass spectrometry
GEF	global evaluation factor
GLP	good laboratory practices
GSH	reduced glutathione
H3P	H3 phosphorylation
HBGV	health-based guidance values
HPLC	high performance liquid chromatography
IC	ion chromatography
ICCR	Institute for Competent Contract Research
ICP–MS	inductively coupled plasma–mass spectrometry
IOFI	International Organization of the Flavor Industry
IQ	intelligence quotient
ISS	Istituto Superiore di Sanità
ITEM	Institute for Toxicology and Experimental Medicine
IWGT	international workshops on genotoxicity testing
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC–MS	liquid chromatography–mass spectroscopy
LDH	lactate dehydrogenase
LOQ	limit of quantification
ML	maximum level
MN	micronucleus
MNHEP	micronucleated hepatocytes
MNIE	micronucleated immature erythrocytes
MNPCE	micronucleated polychromatic erythrocytes
MNT	micronucleus test
MOE	margin of exposure

MS	mass spectrometry
MTD	maximum tolerated dose
NCE	normochromatic erythrocytes
NDMA	N-nitrosodimethylamine
NMR	Nuclear magnetic resonance
OECD	Organisation for Economic Co-operation and Development
P95	95th percentile
PAHs	polycyclic aromatic hydrocarbons
PCE	polychromatic erythrocytes
PCLS	precision cut liver slices
Py-GC–MS	Pyrolysis-gas chromatography–mass spectroscopy
QSAR	quantitative structure–activity relationship
RFU	random fluorescence units
ROS	reactive oxygen species
RP	reference points
RSD	relative standard deviation
SD	standard deviation
SF	smoke flavouring
SOPs	Standard Operating Procedures
TG	test guideline
TGR	transgenic rodent
TI	tail intensity
TK	thymidine kinase
TOF–MS	time of flight–mass spectroscopy
TR	technical requirements
TTC	threshold of toxicological concern
TWI	tolerable weekly intake
UDS	unscheduled DNA synthesis
WOE	weight of evidence
wt	weight

Appendix A – Full list of identified and quantified constituents of the smoke flavouring Primary Product (SF-003)

Table A.1: Compilation of the 129 identified and quantified volatile constituents in the Primary Product (Documentation provided to EFSA No. 1 and 2)

CAS no.	FL-no	Chemical name[a]	Mean amount[b] (FID Area – %)	semi-quantitative IOFI-based result[c] (wt%)	Quantitative result (wt%)[d]
64-19-7	08.002	Acetic acid	7.6	7.6	7.1
498-07-7	–	β-D-glucopyranose, 1,6-anhydro-	4.8	4.2	5.1
91-10-1	04.036	2,6-dimethoxyphenol (phenol, 2,6-dimethoxy-)	10.4	2.0	2.0
116-09-6	07.169	1-hydroxypropan-2-one (2-propanone, 1-hydroxy)	2.3	1.8	0.6
6638-05-7	04.053	4-methyl-2,6-dimethoxyphenol (phenol, 2,6-dimethoxy,-4-methyl-)	7.0	1.3	1.3
141-46-8	–	Acetaldehyde, hydroxy-	0.8	1.2	0.5
10374-51-3	–	2(3*H*)-furanone, dihydro-5- (hydroxymethyl)-	1.3	0.9	0.8
107-21-1	–	1,2-ethanediol (ethylene glycol)	0.8	0.9	0.2
16874-33-2	–	2-furancarboxylic acid, tetrahydro-	1.2	0.9	<0.5
64-18-6	08.001	Formic acid	0.1	0.8	0.9
203506-97-2	–	Threo-pentonic acid, 3-deoxy-, γ-lactone	0.8	0.8	
19444-84-9	–	2(3*H*)-furanone, dihydro-3-hydroxy-	0.8	0.7	
765-70-8	07.056[e]	3-methylcyclopentan-1,2-dione (1,2-cyclopentanedione, 3-methyl)	3.9	0.7	0.7
14059-92-8	04.052	4-ethyl-2,6-dimethoxyphenol (phenol, 4-ethyl-2,6-dimethoxy)	3.9	0.7	0.7
123-76-2	08.023	4-oxovaleric acid (pentanoic acid, 4-oxo-)	0.8	0.6	
120-80-9	04.029	Benzene-1,2-diol (1,2-benzenediol)	2.7	0.5	1.2
58534-89-7	–	3(2*H*)-furanone, dihydro-5- (hydroxymethyl)-	0.5	0.4	
20675-95-0	04.055[f]	2,6-dimethoxy-4-prop-1-enylphenol (phenol, 2,6-dimethoxy-4- (1*E*)-1-propen-1-yl-)	2.1	0.4	0.4
497-23-4	Former 10.066[g]	Furan-2(5*H*)-one (2(5*H*)furanone)	1.6	0.4	0.6
5058-01-5	–	2H-pyran-2-one, tetrahydro-3- hydroxy-	1.5	0.3	0.2

CAS no.	FL-no	Chemical name[a]	Mean amount[b] (FID Area – %)	semi-quantitative IOFI-based result[c] (wt%)	Quantitative result (wt%)[d]
5469-16-9	–	2(3*H*)-furanone, dihydro-4-hydroxy-	0.34	0.33	
13494-08-1	07.057[h]	3-ethylcyclopentan-1,2-dione (1,2-cyclopentanedione, 3-ethyl)	0.69	0.33	
79-09-4	08.003	Propionic acid (propanoic acid)	0.41	0.32	
934-00-9	–	3-methoxycatechol (1,2-benzenediol, 3-methoxy-)	1.48	0.31	0.83
19037-58-2	–	2-propanone, 1-(4-hydroxy-3,5- dimethoxyphenyl)-	1.56	0.29	0.6
5077-67-8	07.090	1-hydroxybutan-2-one (2-butanone, 1-hydroxy)	0.48	0.29	
88-14-2	13.136	2-furoic acid (2-furancarboxylic acid)	0.28	0.26	
6627-88-9	04.051	4-allyl-2,6-dimethoxyphenol (phenol, 2,6-dimethoxy-4-(2-propen-1-yl)-)	1.38	0.24	0.25
67-47-0	13.139	5-hydroxymethylfurfuraldehyde (2-furancarboxaldehyde, 5-(hydroxymethyl)-)	0.95	0.22	0.17
6124-79-4	–	2(5*H*)-furanone, 4-methyl-	0.35	0.22	
2503-46-0	–	2-propanone, 1-(4-hydroxy-3- methoxyphenyl)-	1.09	0.20	0.21
n.a.	–	pentanoic acid, 2,5-dihydroxy-, ethyl ester	0.35	0.20	
29393-32-6	–	2(3*H*)-furanone, 5-acetyldihydro-	0.27	0.18	
118-71-8	07.014	Maltol (4*H*-pyran-4-one, 3-hydroxy-, 2-methyl)	0.82	0.18	0.27
2478-38-8	07.164	4-hydroxy-3,5-dimethoxyacetophenone (ethanone, 1-(4-hydroxy-3,5- dimethoxyphenyl)-)	0.92	0.18	0.28
123-31-9	–	1,4-benzenediol	0.93	0.17	0.18
22122-36-7	–	2(5*H*)-furanone, 3-methyl-	0.27	0.16	
93-51-6	04.007	2-methoxy-4-methylphenol (phenol, 2-methoxy-4- methyl-)	0.96	0.15	0.14
592-20-1	09.185	2-oxopropyl acetate (2-propanone, 1-(acetyloxy)-)	0.20	0.14	
1501-27-5	–	Pentanedioic acid, 1-methyl ester	0.18	0.14	
98-00-0	13.019	Furfuryl alcohol (2-furanmethanol)	0.22	0.13	

CAS no.	FL-no	Chemical name[a]	Mean amount[b] (FID Area – %)	semi-quantitative IOFI-based result[c] (wt%)	Quantitative result (wt%)[d]
90-05-1	04.005	2-methoxyphenol (phenol, 2-methoxy)	0.78	0.13	0.14
5650-43-1	07.154	1-(3,5-dimethoxy-4-hydroxyphenyl)propan-1-one (1-propanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-)	0.65	0.13	0.12
96-48-0	10.006	Butyro-1,4-lactone (2(3*H*)furanone, dihydro)	0.60	0.13	0.3
134-96-3	05.153	4-hydroxy-3,5-dimethoxybenzaldehyde (benzaldehyde, 4-hydroxy- 3,5-dimethoxy-)	0.56	0.12	
6766-82-1	04.056	2,6-dimethoxy-4-propylphenol (phenol, 2,6-dimethoxy-4-propyl)	0.65	0.11	
26624-13-5	04.055[f]	2,6-dimethoxy-4-prop-1-enylphenol (phenol, 2,6-dimethoxy-4- (1*Z*)-1-propen-1-yl-)	0.61	0.11	0.2
3878-55-5	–	Butanedioic acid, 1-methyl ester	0.12	0.11	
591-11-7	–	2(5*H*)-furanone, 5-methyl-	0.17	0.10	
108-95-2	04.041	Phenol	0.67	0.10	
2758-18-1	07.112	3-methyl-2-cyclopenten-1-one (2-cyclopenten-1-one, 3-methyl)	0.22	0.09	
10551-58-3	–	2-furancarboxaldehyde, 5- [(acetyloxy)methyl]-	0.13	0.09	
107-93-7	08.072[i]	But-2-enoic acid (2-butenoic acid, (2*E*)-)	0.13	0.09	
17678-19-2	–	Ethanone, 1-(2-furanyl)-2-hydroxy-	0.12	0.09	
n.a.	–	2-(hydroxymethyl)-5-methoxy-2- methyl-tetrahydrofuran-3-one	0.15	0.09	
98-01-1	13.018	Furfural (2-furancarboxaldehyde)	0.13	0.08	
498-02-2	07.142	Acetovanillone (ethanone, 1-(4-hydroxy-3-methoxyphenyl)-)	0.41	0.08	
121-33-5	05.018	Vanillin (benzaldehyde, 4-hydroxy-3-methoxy-)	0.35	0.08	
13494-07-0	07.076	3,5-dimethylcyclopentan-1,2-dione (1,2-cyclopentanedione, 3,5-dimethyl-)	0.16	0.07	
106-44-5	04.028	4-methylphenol (phenol, 4-methyl-)	0.48	0.07	
1073-96-7	–	4*H*-pyran-4-one, 3,5-dihydroxy-2- methyl-	0.08	0.07	

CAS no.	FL-no	Chemical name[a]	Mean amount[b] (FID Area – %)	semi-quantitative IOFI-based result[c] (wt%)	Quantitative result (wt%)[d]
107-92-6	08.005	Butyric acid (butanoic acid)	0.11	0.07	
108-97-4	–	4H-pyran-4-one	0.10	0.06	
108-39-4	04.026	3-methylphenol (phenol, 3-methyl-)	0.45	0.06	
504-15-4	–	1,3-benzenediol, 5-methyl-	0.36	0.06	
2785-87-7	04.049	2-methoxy-4-propylphenol (phenol, 2-methoxy-4-, propyl-)	0.39	0.06	
452-86-8	–	1,2-benzenediol, 4-methyl-	0.35	0.06	0.42
13494-06-9	07.075	3,4-dimethylcyclopentan-1,2-dione (1,2-cyclopentanedione, 3,4-dimethyl)	0.11	0.06	
930-30-3	–	2-Cyclopenten-1-one	0.13	0.06	
80-59-1	08.064	(2E)-methylcrotonic acid (2-butenoic acid, 2-methyl-,(2E)-)	0.10	0.06	
5932-68-3	04.004[j]	Isoeugenol (phenol, 2-methoxy-4-(1*E*)-1-propen-1-yl-)	0.32	0.05	
95-48-7	04.027	2-methylpheno (phenol, 2-methyl-)	0.38	0.05	
306-08-1	–	Benzeneacetic acid, 4-hydroxy-3- methoxy-	0.26	0.05	
109-00-2	–	3-pyridinol	0.31	0.05	
2785-89-9	04.008	4-ethylguaiacol (phenol, 4-ethyl-2-methoxy-)	0.34	0.05	
884-35-5	–	Benzoic acid, 4-hydroxy-3,5- dimethoxy-, methyl ester	0.22	0.05	
96-35-5	–	Acetic acid, 2-hydroxy-, methyl ester	0.04	0.05	
3008-40-0	–	1,2-cyclopentanedione	0.24	0.05	
1121-05-7	–	2-cyclopenten-1-one, 2,3-dimethyl-	0.12	0.05	
110-13-4	–	2,5-hexanedione	0.09	0.04	
5912-86-7	04.004[j]	Isoeugenol (phenol, 2-methoxy-4-(1*Z*)-1-propen-1-yl-)	0.24	0.04	
3943-74-6	09.799	Methyl vanillate (benzoic acid, 4-hydroxy-3-methoxy-, methyl ester)	0.17	0.04	
104093-74-5	–	3-penten-2-one, 1-hydroxy-	0.06	0.04	
488-17-5	–	1,2-benzenediol, 3-methyl-	0.17	0.03	0.42
1575-46-8	–	2(5*H*)-furanone, 3,4-dimethyl-	0.07	0.03	
1121-07-9	–	2,5-pyrrolidinedione, 1-methyl-	0.05	0.03	
72845-79-5	–	2-propanone, 1-(1-oxopropoxy)-	0.05	0.03	
624-45-3	–	Pentanoic acid, 4-oxo-, methyl ester	0.05	0.03	
123-08-0	05.047	4-hydroxybenzaldehyde (benzaldehyde, 4-hydroxy)	0.15	0.03	

CAS no.	FL-no	Chemical name[a]	Mean amount[b] (FID Area – %)	semi-quantitative IOFI-based result[c] (wt%)	Quantitative result (wt%)[d]
57-55-6	–	1,2-propanediol	0.04	0.03	
97-53-0	04.003	Eugenol (phenol, 2-methoxy-4-(2-propen-1-yl)-)	0.16	0.03	
121-71-1	–	Ethanone, 1-(3-hydroxyphenyl)-	0.15	0.02	
623-50-7	–	Acetic acid, 2-hydroxy-, ethyl ester	0.04	0.02	
33488-51-6	–	2(5*H*)-furanone, 3,4,5-trimethyl-	0.05	0.02	
620-02-0	13.001	5-methylfurfural (2-furancarboxaldehyde, 5-methyl-)	0.04	0.02	
6443-69-2	–	Benzene, 1,2,3-trimethoxy-5-methyl-	0.10	0.02	
65-85-0	08.021	Benzoic acid	0.10	0.02	
944-99-0	–	Phenol, 2,6-dimethoxy-, 1-acetate	0.09	0.02	
623-17-6	13.128	Furfuryl acetate (2-furanmethanol, 2-acetate)	0.02	0.02	
26329-68-0	–	2(5*H*)-furanone, 5-ethyl-3-methyl-	0.04	0.02	
1445-45-0	–	Ethane, 1,1,1-trimethoxy-	0.03	0.02	
1192-62-7	13.054	2-acetylfuran (ethanone, 1-(2-furanyl)-)	0.02	0.01	
2883-45-6	–	1,6-heptadien-4-ol	0.03	0.01	
95-65-8	04.048	3,4-dimethylphenol (phenol, 3,4-dimethyl-)	0.08	0.01	
1003-29-8	Former 14.145[g]	Pyrrole-2-carbaldehyde (1*H*-pyrrole-2-carboxaldehyde)	0.02	0.01	
123-07-9	04.022	4-ethylphenol (phenol, 4-ethyl-)	0.08	0.01	
105-67-9	04.066	2,4-dimethylphenol (phenol, 2,4-dimethyl-)	0.07	0.01	
494-99-5	–	Benzene, 1,2-dimethoxy-4-methyl-	0.07	0.01	
97-99-4	13.020	Tetrahydrofurfuryl alcohol (2-furanmethanol, tetrahydro-)	0.02	0.01	
3008-43-3	07.080	3-methylcyclohexan-1,2-dione (1,2-cyclohexanedione, 3-methyl-)	0.02	0.01	
83-33-0	–	1H-inden-1-one, 2,3-dihydro-	0.06	0.01	
513-86-0	07.051	3-hydroxybutan-2-one (2-butanone, 3-Hydroxy)	0.01	0.01	
90-00-6	04.070	2-ethylphenol (phenol, 2-ethyl-)	0.04	0.01	

CAS no.	FL-no	Chemical name[a]	Mean amount[b] (FID Area – %)	semi-quantitative IOFI-based result[c] (wt%)	Quantitative result (wt%)[d]
526-75-0	04.065	2,3-dimethylphenol (phenol, 2,3-dimethyl-)	0.05	0.01	
620-17-7	04.021	3-ethylphenol (phenol, 3-ethyl-)	0.05	0.01	
23074-10-4	–	2-furancarboxaldehyde, 5-ethyl-	0.01	0.01	
576-26-1	04.042	2,6-dimethylphenol (phenol, 2,6-dimethyl)	0.028	0.004	
7295-76-3	–	Pyridine, 3-methoxy-	0.021	0.004	
431-03-8	07.052	Diacetyl (2,3-butandione)	0.006	0.004	
111-55-7	–	1,2-ethanediol, 1,2-diacetate	0.005	0.003	
1453-62-9	–	Furan, 2-(dimethoxymethyl)-	0.004	0.002	
634-36-6	04.084	1,2,3-trimethoxybenzene (benzene, 1,2,3-trimethoxy-)	0.012	0.002	
18402-90-9	–	3,5-heptadien-2-one, (3E,5E)-	0.005	0.002	
1193-18-6	07.098	3-methylcyclohex-2-en-1-one (2-cyclohexen-1-one, 3-methyl-)	0.006	0.002	
932-66-1	–	1-acetyl-1-cyclohexene (ethenone, 1-(1-cyclohexen-1-yl)-)	0.004	0.002	
110-86-1	Former 14.008[g]	Pyridine	0.01	0.001	
109-06-8	14.134	2-methylpyridine (pyridine, 2-methyl)	0.009	0.001	
496-78-6	–	Phenol, 2,4,5-trimethyl-	0.006	0.001	
589-93-5	–	Pyridine, 2,5-dimethyl-	0.005	0.001	

wt: weight; IOFI: International Organization of the Flavor Industry; FID: flame ionisation detector.

(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): Represents the mean amounts present in the solvent-free volatile fraction of the Smoke Concentrate 809045.

(c): Semi-quantifications based on GC-FID area – %, applying relative GC response factors predicted according to a modified mathematical model developed by IOFI. Values represent means determined by analysis of 12 batches of the Primary Product.

(d): Quantifications based on GC–MS/MS, LC–MS, IC and GC/MS, respectively, using external calibrations. The values presented are average values, calculated from the 12 batches listed in Table 7.

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

(f): [FL-no: 04.055] refers to the mixture of *E/Z* stereoisomers of 2,6-dimethoxy-4-prop-1-enylphenol.

(g): '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.

(h): [FL-no: 07.057] refers to the mixture of the tautomeric forms of 3-ethylcyclopentan-1,2-dione.

(i): [FL-no: 08.072] refers to the mixture of *E/Z* isomers of but-2-enoic acid.

(j): [FL-no: 04.004] refers to the mixture of *E/Z* isomers of isoeugenol.

Appendix B – Genotoxicity data available on 16 individual components either from the literature or from experimental studies

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

i) two components for which a concern for genotoxicity has been identified, i.e. furan-2(5*H*)-one and 1,2-benzenediol;
ii) eleven substances listed in Table 12 for which a potential concern for genotoxicity has been identified; and
iii) three components for which the applicant identified a concern for genotoxicity based on literature and/or *in silico* data, i.e. 1-acteyl-1-cyclohexane, pyrrole-2-carbaldehyde, 5-acetyldihydro-2(3*H*)-furanone (see Table 16), and for which additional studies provided by the applicant ruled out the concern for genotoxicity.

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

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[16]). In the evaluation by CoE,[17] 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[18] have been evaluated more recently by ECHA (ECHA, 2016), leading to a standardised classification for genotoxicity as 'Muta 2' for this substance.[19]

Conclusion: Based on experimental *in vitro* and *in vivo* data on benzene-1,2-diol (including studies where animals were exposed via oral route) a concern for genotoxicity *in vivo* is identified. A safety concern emerges since the exposure to benzene-1,2-diol exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 18).

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

Furan-2(5*H*)-one (former [FL-no: 10.066]) was evaluated as genotoxic *in vivo* (EFSA FAF Panel, 2019). To further assess the *in vivo* genotoxicity, furan-2(5*H*)-one was tested in an *in vivo* MN assay in liver of rats (summary of study results reported in Appendix D). In addition, the systemic exposure to furan-2(5*H*)-one was investigated in a satellite group of animals (Documentation provided to EFSA No. 1).

Two additional studies were also performed by the applicant on furan-2(5*H*)-one (Documentation provided to EFSA No. 4). These three new studies were assessed by the Panel as described below.

In vivo micronucleus assay in the liver

Furan-2(5*H*)-one (batch: NEWJF; purity: 94.3%) was tested in an *in vivo* liver micronucleus assay in rats (LSIM, 2022a) which was performed in compliance with GLP and following a validated protocol recommended by the international workshops on genotoxicity testing (IWGT) (Hamada et al., 2015; Uno et al., 2015; Kirkland et al., 2019). An OECD test guideline for the *in vivo* MN study in liver is not yet available. The stability of test article formulations (5 and 20 mg/mL) for up to 8 days was confirmed through a validated analytical method (HPLC) for the determination of furan-2(5*H*)-one in corn oil (0.5–20 mg/mL) (LSIM, 2021).

A dose range finding study was performed to identify the appropriate maximum dose level for the *in vivo* liver micronucleus assay. Groups of three Crl:CD(SD) male rats (6 weeks old at the beginning of dosing) were administered furan-2(5*H*)-one (batch: NEWJF) via oral gavage at 50, 100 and 200 mg/kg bw per day (vehicle control: corn oil) for 14 consecutive days. There were no abnormal clinical signs in

[16] 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. OJ L 180, 19.7.2000, pp. 8–16.
[17] https://book.coe.int/en/health-protection-of-the-consumer/2208-chemically-defined-flavouring-substances.html
[18] 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).
[19] https://echa.europa.eu/da/information-on-chemicals/cl-inventory-database/-/discli/details/67398

any of the groups, but in the top-dose group suppression of bw gain was observed. Therefore, 200 mg/kg bw per day was considered the MTD (LSIM, 2022b).

In the *in vivo* micronucleus assay, groups of five Crl:CD(SD) male rats (6 weeks old at the beginning of dosing) were administered furan-2(5*H*)-one via oral gavage at doses of 0 (vehicle control: corn oil), 50, 100 and 200 mg/kg bw per day for 28 consecutive days. A group of three animals dosed with 12.5 mg/kg bw per day diethyl nitrosamine, administered as per the test item, was used as the positive control (LSIM, 2022a).

Twenty-four hours after the final administration, liver cells were sampled and prepared for the micronuclei analysis for all animals. For the micronuclei analysis, 4,000 hepatocytes per animal (excluding cells in M-Phase) were scored for the presence of micronucleated hepatocytes (MNHEPs). The number of hepatocytes in M-phase was recorded separately and used to calculate the mitotic index.

In order to confirm the systemic exposure of the test item, this study also included a toxicokinetic (TK) analysis of furan-2(5*H*)-one (batch: NEWJF) in plasma for a satellite group of rats (LSIM, 2022a). Groups of three Crl:CD(SD) male rats were treated via oral gavage with furan-2(5*H*)-one at doses of 0 (vehicle control: corn oil) or 200 mg/kg bw per day for 28 consecutive days. Furan-2(5*H*)-one concentrations in rat plasma were analysed via LC–MS/MS using a validated method (LSIM, 2022c). The lower limit of quantification was 100 ng/mL and the upper limit of quantification was 10,000 ng/mL; the calibration curve showed linearity between 100 ng/mL and 10,000 ng/mL (LSIM, 2022c).

In the top-dose group of the main study, one animal showed slight salivation 1 h after dosing on Day 23 and Day 24. No clinical signs were observed in any other animals of any group. Also in the top-dose group, there were significant decreases in bw from Day 15 onwards and significant increases in relative liver weight compared to the negative control.

The acceptance criteria of the study were fulfilled. A statistically significant increase in micronucleated hepatocytes (MNHEP) with respect to the concurrent vehicle control was reported in positive control group, which was within the range of laboratory's historical positive control data (based only on 16 animals, number of experiments not reported). The vehicle control data were within the range of laboratory's historical vehicle control data (based on 68 animals, number of experiments not reported).

A statistically significant and dose-dependent increase in MNHEPs, compared with the concurrent vehicle control group was detected in 100 and 200 mg/kg bw per day test compound groups. At 200 mg/kg bw per day the increase (nine-fold the values in the control animals) in MNHEPs exceeded the range of historical negative control data (min – max, 0.00–0.25%).

The mitotic index in the liver was not affected by the treatment with furan-2(5*H*)-one at any dose level.

In the TK analysis, Tmax occurred at 0.5 h after both the initial and final administrations. The Cmax and AUC0-24 h values at the final dosing were lower than at the initial dosing, but systemic exposure was confirmed throughout the dosing period. Salivation was noted in all furan-2(5*H*)-one-treated animals on various days and bw gain was lower compared to the vehicle control group.

According to the study authors, satisfactory linearity, recovery and repeatability were found for furan-2(5*H*)-one when the substance was spiked and analysed in rat plasma samples. However, the Panel noted that linearity in plasma extracts was in the range of 100–10,000 ng/mL, but the concentration reported for furan-2(5*H*)-one in rat plasma samples was above this range after the initial dosing, i.e. Cmax 15,060 ng/mL. Moreover, the recovery and accuracy of the method were only determined for the ranges 200–8,000 ng/mL and 100–8,000 ng/mL respectively.

The study authors concluded that the study is valid and that furan-2(5*H*)-one has the potential to induce micronuclei in rat liver hepatocytes *in vivo* under the conditions of this study. The Panel concurred with this finding. The Panel considered the study as reliable without restrictions and the results of high relevance (see Appendix D, Table D2).

Based on the positive results observed in the *in vivo* MN study, the applicant submitted a study to investigate the pro-oxidative potential of furan-2(5*H*)-one and an *in vitro* MultiFlow® screen with the aim of clarifying the MOA and to determine whether the genotoxic effect observed is threshold-mediated.

Pro-oxidative Potential of furan-2(5*H*)-one

An exploratory study was conducted to investigate the potential of furan-2(5*H*)-one to elevate concentrations of reactive oxygen species (ROS) in two different *in vitro* liver models: human hepatoblastoma (HepG2) cells and precision cut liver slices (PCLS) obtained from healthy (nulliparous

and non-pregnant) Wistar rats (strain Crl:WI (Han)) (Fraunhofer ITEM, 2023). The PCLS model consists of different cell types, including immune cells and therefore can include assessment of ROS production resulting from inflammatory processes.

Furan-2(5*H*)-one (batch: BCCD2607; purity: 98.6%) was tested according to the relevant Standard Operating Procedures (SOPs) of the Fraunhofer ITEM and, for the two *in vitro* comet assay pilot experiments, also in line with the principles suggested in OECD TG 489 (OECD, 2016d) regarding the most appropriate measure for DNA damage following analyses of single cells. According to the study report, the study was conducted in the spirit of the basic requirements of GLP.

Prior to ROS measurements, a cytotoxicity screen was performed in HepG2 cells over a concentration range of 15.6–500 μg/mL furan-2(5*H*)-one for 3 h in the absence and presence of S9-mix and for 24 h without S9-mix. Cytotoxicity was determined using cell morphology and cell density for 24 h in the absence of S9-mix only (i.e. light microscopy), cell proliferation/cell loss (i.e. automatic cell counting) for all treatments, membrane damage (i.e. lactate dehydrogenase (LDH) release assay) 3 h and 24 h in the absence of S9-mix only, and metabolic activity (i.e. WST-1 assay) for all treatments. Triton™ X-100 (0.1% [v/v]) served as positive control and DMSO (0.8% [v/v]) as vehicle control. Three separate experiments each in triplicate were performed. Significant furan-2(5*H*)-one cytotoxicity was only evident after 24 h exposures and mainly at concentrations higher than 62.5 μg/mL, with steep increases observed between 62.5 and 125 μg/mL in the WST-1 and LDH release assays. The S9-mix had no considerable impact on the induction of cytotoxicity by furan-2(5*H*)-one as measured using the WST-1 assay. Subsequent studies to assess ROS were performed at higher concentrations than applied in this cytotoxicity study in which the authors attempted to determine if ROS could be a potential MOA in these cytotoxicity screening assays.

For the ROS studies, HepG2 cells were exposed to five different concentrations (0, 125, 250, 500 and 1,000 μg/mL) of furan-2(5*H*)-one for 3 h or 24 h exposures with or without reduced glutathione (GSH, 5 mM, approximately 1,537 μg/mL). Luperox® tert-butyl hydroperoxide (TBH70X) was used as a positive control (1 mM, approximately 242 μg/mL) and 0.8% DMSO was used as negative/vehicle control. ROS-dependent intracellular 2′,7′-dichloro-dihydro-fluorescein (DCFH) fluorescence was measured at 60, 120 and 180 mins. Velocity of ROS generation was subsequently calculated considering the linear increase in random fluorescence units (RFU)/time between 60 mins and 180 mins. Three separate experiments each in triplicate were performed. In other studies, rat PCLS were exposed to four different concentrations (0, 250, 375 and 500 μg/mL) of furan-2(5*H*)-one for 4 h exposures with or without GSH (5 mM, approximately 1,537 μg/mL). TBH7OX (10 mM, approximately 2,422 μg/mL) was used as positive control and 0.4% DMSO was used as negative/vehicle control. ROS-dependent fluorescence of intracellular chloromethyl-2′,7′-dichloro-dihydro-fluorescein (CM-H2DCF) was measured after 240 mins. Three separate experiments each in duplicate were performed. A sample of PCLS-treated media was also obtained for the determination of LDH release.

In HepG2 cells, furan-2(5*H*)-one induced a slight increase of ROS at 500 and 1,000 μg/mL following 3 h exposures, reaching statistical significance only at the highest concentration. This significant increase was partially, but statistically significantly, counteracted by GSH. Following a 24 h co-exposure with furan-2(5*H*)-one and GSH, relative cell counts were decreased and LDH release increased for the +GSH control compared to the -GSH control. At 250 and 500 μg/mL (and also at 1,000 μg/mL in the case of LDH release), the effects of furan-2(5*H*)-one were partially and significantly counteracted by GSH ($p \leq 0.01$–$p \leq 0.001$).

In rat PCLS, 4 h furan-2(5*H*)-one exposures induced a slight increase of ROS at 250 μg/mL, which was increased at 375 μg/mL and at both concentrations completely counteracted by GSH when compared to the negative control +GSH. At the highest concentration (500 μg/mL of furan-2(5*H*)-one), no significant increase in ROS was observed. LDH release showed similar patterns as for ROS production, but GSH treatment had a lesser counteractive effect.

To determine the clastogenic potential of furan-2(5*H*)-one in HepG2 cells, pilot alkaline comet assays in HepG2 cells were performed after 3 h exposures with furan-2(5*H*)-one at concentrations of 0, 62.5, 125, 250 and 500 μg/mL with or without concomitant addition of GSH (5 mM, approximately 1,537 μg/mL). Ethyl methanesulfonate (EMS, 0.75 μL/mL, 1 h) was used as positive control for induction of DNA strand breaks and DMSO (0.4% [v/v]) was used as vehicle control. Three biological replicates were analysed. A slight (but statistically significant) increase in mean tail intensity (TI) was evident at all concentrations that was maximal at 125 μg/mL (mean TI 1.82 ± 0.31% vs. 0.45 ± 0.04% for negative control without GSH), but it was not concentration-dependent. GSH treatment completely (62.5–250 μg/mL) or partially (500 μg/mL; mean TI without GSH: 1.79 ± 0.55%

vs. mean TI with GSH: 1.14 ± 0.37%) inhibited effects as compared to the negative control +GSH (mean TI: 0.69 ± 0.160%).

Additionally, an acellular comet assay was performed using lysed L5178Y/TK$^{+/-}$ mouse lymphoma cells as a DNA source as no cell-type specific functions were needed for the assay. This assay was performed to determine the direct DNA-damaging potential of furan-2(5*H*)-one without cellular enzymes such as those for metabolic activation or DNA repair. Furan-2(5*H*)-one was tested at concentrations of 0, 125, 250 and 500 μg/mL for a 1 h exposure. With EMS (1 μL/mL, 1 h) single-strand breaks were observed. DMSO (0.4% [v/v]) was used as vehicle control. Four biological replicates were analysed. TI was used as a measure of DNA strand breakage. In this acellular comet assay, furan-2(5*H*)-one had no effect at any concentration.

Overall, furan-2(5*H*)-one was able to induce oxidative stress in the two liver cell models, and as the slight clastogenic potential of furan-2(5*H*)-one in HepG2 cells was effectively counteracted by GSH, induction of ROS might, therefore, be at least partly responsible for the slight clastogenic activity. However, the exact mechanism(s) and types of ROS generated, as well as cell type-specificity, remain to be specified. From this information, the study author considered that clastogenicity in the presence of oxidative stress may be consistent with a non-DNA-reactive MOA, which is expected to exhibit a threshold, i.e. at concentrations below which physiological stress/significant cytotoxicity is caused, no genotoxic effects will occur.

The Panel concluded that, whilst it is possible that ROS contributed to the responses seen at relatively high concentrations in the comet assays with HepG2 cells, it is not possible to confirm from the available evidence that this MOA contributes to the genotoxicity of furan-2(5*H*)-one. In particular, the potential of ROS production to be a cause or a consequence of cytotoxicity is not clear and the mode(s) of action of the inhibitory effect of GSH on reducing ROS concentrations, comet responses and cell toxicity has not been demonstrated. The fact that GSH can protect against the effect of a direct acting genotoxicant as well as a pro-oxidant is evident from the result of the positive control agent. Further to these considerations, it should also be noted that in HepG2 a clastogenic effect was observed at concentrations well below those inducing a significant production of ROS (62.5 vs. 1,000 μg/mL). There is no specific evidence of ROS-induced DNA lesions or on absence of direct reaction of furan-2(5*H*)-one with DNA.

In Vitro MultiFlow® Screen

To evaluate genotoxic potential, with a focus on MOA (i.e. clastogenicity and aneugenicity), furan-2(5*H*)-one (batch: BCCD2607, purity 98.6%) was tested in an *in vitro* MultiFlow® Assay using TK6 human lymphoblasts, both in the absence and in the presence of induced rat liver S9 fraction (S9-mix) (Charles River, 2023). A panel of nuclear biomarkers that have been shown to distinguish between aneugenicity and clastogenicity mechanisms was used: γH2AX, H3 phosphorylation (H3P), polyploidisation and p53 translocation (Bryce et al., 2018). Cleaved-PARP was also tested as an indicator of the health of the cultures. As there is no OECD TG for this method, the assay was carried out in accordance with the MultiFlow® DNA Damage Kit's manual (Litron Laboratories; Rochester, US). This study is not GLP. The study report only provided a protocol with limited and sometimes contradictory summary, but results were adequately reported.

There were four positive controls tested at four concentrations each: methyl methanesulfonate (12.5, 25, 50 and 100 μM), carbendazim (12.5, 25, 50 and 100 μM), benzo(a)pyrene (1.25, 2.5, 5 and 10 μM) and cyclophosphamide (2.5, 5, 10 and 20 μM). DMSO was used as vehicle control. In absence of metabolic activation, all test item concentrations and positive controls were evaluated after 4 h or 24 h of exposure. In presence of metabolic activation, the exposure lasted for 4 h followed or not by a 20 h expression period and for 24 h continuous exposure with and without S9-mix. The test item and positive controls were evaluated in single replicate cultures whilst the vehicle control was evaluated in eight replicate cultures.

The highest concentration for all exposure conditions was 842 μg/mL (approximately 10 mM) furan-2(5*H*)-one, which was reported to be freely soluble at the end of both exposure periods. At the end of the 4 h and 24 h exposure periods, an aliquot from each culture was taken, placed in lysis solution and analysed using flow cytometry with FACSDiva software. Cytotoxicity was calculated based upon relative nuclei counts. The highest furan-2(5*H*)-one concentrations evaluated for genotoxicity were those inducing ≤ 80% cytotoxicity. In the absence of S9-mix, furan-2(5*H*)-one was tested at 11 concentrations ranging from 12.1–113 μg/mL (74% cytotoxicity at 24 h) and in the presence of S9-mix was tested at 12 concentrations ranging 12.1–141 μg/mL (64% cytotoxicity at 24 h).

Clastogenicity and aneugenicity were assessed using independent machine learning models developed using JMP Pro statistical software (v12.2.0). Clastogenicity signatures were demonstrated by either two consecutive concentrations with clastogenic probability scores ≥ 80% or one concentration with a probability score ≥ 90% and conversely, aneugenic signatures were demonstrated by either two consecutive concentrations with aneugenic probability scores ≥ 80% or one concentration with a probability score ≥ 90%. For any test concentration to be considered as having clastogenic or aneugenic signatures two of the three models needed to be in agreement. In addition, global evaluation factors (GEFs) were created using JMP statistical software (v12.2.0) and applied. Clastogenicity signatures were demonstrated by fold increases in two consecutive concentrations that met or exceeded cut-offs for at least two of the following clastogenic responses: ≥ 1.51-fold 4 h γH2AX, ≥ 2.11-fold 24 h γH2AX, ≥ 1.40-fold 4 h nuclear p53 and ≥ 1.45-fold 24 h nuclear p53. Conversely, aneugenic signatures for cultures without S9-mix were demonstrated by fold increases in two consecutive concentrations that met or exceeded cut-offs for at least two of the following aneugenic responses: ≥ 1.71-fold 4 h H3P+ nuclei, ≥ 1.52-fold 24 h H3P+ nuclei, ≥ 5.86-fold 24 h polyploidy and ≥ 1.45-fold 24 h nuclear p53.

For with S9-mix cultures, only the clastogen MOA was investigated. Clastogenicity MOA probability scores were assessed using independent machine learning models as described above. In addition, GEFs were created as described above. Clastogenicity signatures were demonstrated by fold increases in two consecutive concentrations that met or exceeded cut-offs for at least two of the following clastogenic responses (at least one being γH2AX): ≥ 1.44-fold 4 h γH2AX, ≥ 1.31-fold 24 h γH2AX, ≥ 1.23-fold 4 h nuclear p53 and ≥ 1.12-fold 24 h nuclear p53.

In the absence of S9-mix, from the machine learning models there was a prediction of a clastogenic but not aneugenic signature at test concentrations ≥ 90.4 μg/mL. Also, at the same concentration statistically significant increases in γH2AX and p53 translocation were observed at 24 h, exceeding the respective GEFs. In the presence of S9-mix, there were no statistically significant increases in markers for clastogenicity observed at any concentration evaluated based upon the machine learning models. Nevertheless, statistically significant increases, exceeding their respective GEFs, were observed for γH2AX at 4 h and p53 translocation at 4 h and 24 h at concentrations ≥ 113 μg/mL, which would indicate a clastogenic potential.

The study author concludes that the results indicate that furan-2(5*H*)-one was not aneugenic in absence of metabolic activation and that it was clastogenic, both in the absence and in the presence of S9-mix, under the conditions of the study.

Despite the fact that the study methodology is not validated, the Panel recognises that the study results provide further support for the clastogenic properties of furan-2(5*H*)-one.

Conclusion: the positive results from previously available studies (see EFSA FAF Panel, 2019) as well as from the newly submitted *in vivo* MN assay in liver and the positive results obtained by the newly submitted *in vitro* MultiFlow® test on TK6 cells support a clastogenic MOA for furan-2(5*H*)-one. Evidence to suggest that the genotoxicity of furan-2(5*H*)-one is mediated through ROS production is inadequate. Therefore, a safety concern emerges for this component, since the exposure to furan-2(5*H*)-one exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 18).

B.3. 3-Methoxycatechol (CAS No. 934-00-9)

The applicant performed the following two *in vitro* genotoxicity studies on 3-methoxycatechol, which were assessed by the Panel as described below.

Bacterial reverse mutation assay

A bacterial reverse mutation assay was conducted in *S*. Typhimurium strains TA98, TA100, TA1535, TA1537 and in *E. coli* WP2 uvrA to assess the mutagenicity of 3-methoxycatechol (batch G8303/0; purity 98.8%), both in the absence and in the presence of metabolic activation by phenobarbital/β-naphthoflavone-induced rat liver S9 fraction (S9-mix) (ICCR, 2022a). The study complies with OECD TG 471 (OECD, 2020b) and with GLP principles.

In the first experiment, conducted using the plate incorporation method, 3-methoxycatechol was tested up to a maximum concentration of 5,000 μg/plate, with and without S9-mix. Evident toxicity was reported in the absence of S9-mix at relatively high concentrations. In this experiment a 2.1-fold increase in the mean number of revertant colonies was observed in strain TA100 in the presence of S9-mix, only at 5,000 μg/plate. This increase was slightly over the historical control range.

Furthermore, strain TA98 without S9-mix showed a minor increase (1.8-fold) in the mean number of revertant colonies that however remained within the historical control range.

A second experiment was conducted using the pre-incubation method. In the presence of S9-mix the maximum concentration tested was 5,000 μg/plate. In the absence of S9-mix, due to the toxicity reported in the first experiment, 3-methoxycatechol was tested up to 2,500 μg/plate. In this experiment, for strain TA100 with S9-mix the increase in the mean number of revertant colonies was less than two-fold and remained within historical solvent control values. No increase was observed in any other strain.

Two confirmatory experiments were performed only in strain TA100 with S9-mix, using the plate incorporation and the pre-incubation methods. Slight increases (less than 1.7-fold) were reported in both experiments, but the values were always within the historical control range. An apparent trend in the concentration response for mutation frequency was observed in all four experiments with TA100 in the presence of S9-mix.

In all experiments, positive control chemicals both with and without S9-mix induced significant increases in revertant colony numbers and both vehicle and positive controls were within the respective historical control ranges.

In summary, in TA100 in the presence of S9-mix, 3-methoxycatechol induced an increase (2.1-fold) in mutation frequency compared to the control level at the top concentration in one experiment. The increase was slightly above the historical control range. In three other experiments it induced slight increases (<two-fold) in revertants frequency, but all these increases were within the historical control range. Although there is an apparent trend in the concentration response for mutation frequency with TA100 in the presence of S9-mix, the level of increase was variable and low. Consequently, the biological relevance of the observed weak effect is questionable and the results are considered equivocal. The Panel evaluated the study as reliable without restrictions, but the equivocal results are considered of limited relevance.

In vitro mammalian cell micronucleus test

An *in vitro* micronucleus assay, with cytokinesis block protocol, was carried out in accordance with OECD TG 487 (OECD, 2016e) and GLP. Human peripheral blood lymphocytes from healthy donors were treated with 3-methoxycatechol (batch: G8303/0; purity 98.8%). Positive controls were cyclophosphamide, mitomycin C and vinblastine. Deionised water was used as vehicle control. Two separate experiments were carried out: in the first experiment 3 h exposures with and without metabolic activation (liver fraction (S9-mix) from rats induced by phenobarbital/β-naphthoflavone) were applied, in the second experiment a 28 h exposure without S9-mix was applied. For each experimental condition, two cultures were analysed in parallel (ICCR, 2022b).

The highest concentration tested in the cytotoxicity range-finder experiment using 3 h exposures with and without S9-mix was 1,419 μg/mL (approximately 10 mM). The concentrations used in this experiment were considered appropriate and therefore the data could be used for micronuclei evaluation (experiment 1). In this experiment, lymphocytes were treated with 10 concentrations of 3-methoxycatechol, ranging from 9.2 to 1,419 μg/mL, in the 3 h treatments in both the absence and in the presence of S9-mix. In experiment 2, 11 concentrations, from 3.3 to 200 μg/mL, were tested in the 28 h treatment in the absence of metabolic activation. No precipitate of the test item was noted in any of the exposure conditions.

The cytokinesis-block proliferation index (CBPI) cytotoxicity data were used to select the concentrations for the (MN) analysis.

In the treatment of 3 h + 25 h in the absence of S9-mix, the following concentrations were chosen for MN analysis: 28.2, 49.4 and 86.5 μg/mL (cytotoxicity of 27.1%, 40.6% and 48.6%, respectively).

In the treatment of 3 h + 25 h in the presence of S9-mix, the following concentrations were chosen for MN analysis: 16.1, 49.4 and 151 μg/mL (cytotoxicity of 14.2%, 35.7% and 59.5%, respectively).

In the treatment of 28 h in the absence of S9-mix, the following concentrations were chosen for MN analysis: 17.6, 30.8 and 70 μg/mL (cytotoxicity of 21.1%, 33.0% and 57.2%, respectively).

In all three test conditions, 3-methoxycatechol did not induce statistically significant increases in the frequency of micronucleated cells compared to vehicle controls nor concentration-dependent trends. Therefore, the Panel concluded that 3-methoxycatechol did not induce micronuclei in human peripheral blood lymphocytes in this study. The Panel evaluated the study as reliable without restrictions and the results of high relevance.

Results of the *in vitro* studies on 3-methoxycatechol are summarised in Appendix D, Table D.1.

Conclusion: Given the equivocal results of the bacterial gene mutation assay, an *in vitro* follow-up study would be needed to evaluate the genotoxic potential of the substance, since exposure to 3-methoxycatechol exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 18).

B.4. Derivatives of furan-2(5*H*)-one

The Panel considered that six derivatives of furan-2(5*H*)-one are present in the Primary Product, i.e. 2(5*H*)-furanone, 5-methyl- (CAS No. 591-11-7,); 2(5*H*)-furanone, 3-methyl- (CAS No. 22122-36-7); 2 (5*H*)-furanone, 5-ethyl-3-methyl- (CAS No. 26329-68-0)); 2(5*H*)-furanone, 3,4,5-trimethyl- (CAS No. 33488-51-6); 2(5*H*)-furanone, 3,4-dimethyl- (CAS No. 1575-46-8); 2(5*H*)-furanone, 4-methyl- (CAS No. 6124-79-4).

The Panel noted that these components are structurally related to furan-2(5*H*)-one [former FL-no: 10.066] and to the 2-(5*H*)-furanone-derivative 3,4-dimethyl-5-pentylidenefuran-2(5*H*)-one [FL-no: 10.042]. For 2-(5*H*)-furanone a concern for genotoxicity was identified and for [FL-no: 10.042] a concern for structural and numerical chromosomal aberrations could not be ruled out in FGE.217Rev2 (EFSA FAF Panel, 2019).

For one of these components, i.e. 2(5*H*)-furanone, 4-methyl- (CAS No. 6124-79-4), the Panel noted that a negative Ames test is available in the literature (Lalonde et al., 1991). However, the Panel considered the reliability of this study as insufficient and as of low relevance, due to inadequate study design (only one strain of *S*. Typhimurium (TA100) was used and it was conducted only without metabolic activation) and incomplete reporting.

Conclusion: a potential concern for genotoxicity is identified for these six derivatives of 2(5*H*)-furanone considering the read-across from the above mentioned structurally related substances. Considering the data presented in FGE.217Rev2 for these substances, information on clastogenic and aneugenic potential would be needed to evaluate their genotoxic potential, since the exposures to these components exceed the TTC for DNA-reactive mutagens and/or carcinogens (see Table 18).

B.5. Hydroxyacetaldehyde (CAS No. 141-46-8)

The applicant identified in the literature the following papers reporting studies on hydroxy acetaldehyde.

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

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

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

A bacterial reverse mutation assay was also applied in the study of Pool et al. (1986), which reported a positive result. The insufficient information regarding the methods (*S*. Typhimurium strain/s, compound concentrations) and the results, which are only described as positive or negative, does not allow the evaluation of the reliability of this study.

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

Hussain and Osterman-Golkar (1984) tested the substance in a bacterial reverse mutation assay reporting a concentration-dependent increase of revertants. The only bacterial strain used was the Escherichia coli Sd-4 which was rarely used in the past and is not among those suggested in the OECD TG 471 (OECD, 2020b). Due to the single strain used and the poor reporting, the reliability of the study is insufficient and the relevance is low.

Conclusion: Given the reactivity of the substance towards DNA and the equivocal results of a bacterial gene mutation assay, *in vitro* follow-up 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 18).

B.6. 2-(Hydroxymethyl)-5-methoxy-2-methyl-tetrahydrofuran-3-one (CAS No. not available)

The Panel noted that no relevant information on genotoxicity from the literature has been submitted by the applicant on this substance. However, based on the (Q)SAR analysis performed by EFSA a weak indication for potential genotoxicity was identified (see Annex A).

The Panel investigated whether tetrahydrofuran-derivatives in FGE.75 (EFSA CEF Panel, 2016) and FGE.33 (EFSA, 2008) could be considered as structurally related, but concluded that they only partially reproduce the chemical environment (i.e. the oxolane, the alkyl aldehyde precursor and the ketone group in the oxolane ring) of the target substance. Thus, the Panel concluded that the genotoxicity concern raised by the structural alerts identified by the (Q)SAR analysis cannot be ruled out by read-across considerations.

Conclusion: The Panel concluded that the (Q)SAR analysis provides a weak indication for potential genotoxicity of this constituent. In absence of any experimental data either on the substance or on structurally related substances, appropriate *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 exposure to 2-(hydroxymethyl)-5-methoxy-2-methyl-tetrahydrofuran-3-one exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 18).

B.7. 4H-pyran-4-one (CAS No. 108-97-4)

The applicant performed the following two *in vitro* genotoxicity studies on 4H-pyran-4-one, which were assessed by the Panel as described below.

Bacterial reverse mutation assay

4H-Pyran-4-one (batch: G8304/0; purity 99.2%) was tested in a bacterial reverse mutation test according to the OECD TG 471 (OECD, 2020b) and in compliance with GLP.

Four strains of *S*. Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2*uvrA* pKM101 were used both in the absence and in the presence of metabolic activation by phenobarbital/ β-naphthoflavone-induced rat liver S9 fraction (S9-mix).

Three separate experiments in triplicate were conducted. In Experiment 1, the plate incorporation method was used and in Experiments 2 and 2a the pre-incubation method was used (ICCR, 2022e). Experiment 2a tested strain TA98 with S9-mix only, as in Experiment 2 the negative and solvent control results for this strain were outside the test range of historical values and therefore the results for this strain were invalid. Positive control chemicals and deionised water, as vehicle control, were evaluated concurrently. All tests were evaluated in triplicate plates.

4H-Pyran-4-one was tested at eight concentrations ranging from 3 to 5,000 μg/plate in Experiment 1 and at six concentrations ranging from 33 to 5,000 μg/plate in Experiments 2 and 2a. No precipitation was observed in any experiment.

In Experiments 1 and 2, plates incubated with the test item showed normal background growth for all strains with and without S9-mix. No toxic effects occurred for any test condition.

In Experiment 1, for strain TA 100 without S9-mix an increase (exceeding the two-fold threshold) in the mean number of revertant colonies was observed at 2,500 μg/plate and higher, which also exceeded the maximum value of the historical vehicle controls. For strain TA100 with S9-mix, there was also an increase in the mean number of revertant colonies observed at 5,000 μg/plate but this did not exceed the two-fold threshold nor the limits of the historical vehicle controls. In Experiment 2, there was an increase in the mean number of revertant colonies for strain TA100 without S9-mix (at

1,000 μg/plate and higher) and also with S9-mix (at 5,000 μg/plate) that in both cases exceeded a two-fold increase and also the maximum value of the historical vehicle controls.

The Panel concluded that 4H-pyran-4-one induced gene mutations in bacteria in this study. The Panel considered the study to be reliable without restrictions and its result of high relevance.

***In vitro* mammalian cell micronucleus test**

4H-Pyran-4-one (batch: G8304/0; purity 99.2%) was tested in an *in vitro* micronucleus assay with cytokinesis block protocol, according to OECD TG 487 (OECD, 2016e) and in compliance with GLP. Two separate experiments were carried out in duplicate in human peripheral blood lymphocytes: 3 h exposures with and without metabolic activation by phenobarbital/β-naphthoflavone-induced rat liver S9 fraction (S9-mix) were applied in Experiment 1 and a 28 h exposure without S9-mix was applied in Experiment 2 (ICCR, 2022f).

A range-finder experiment using 3 h exposures with and without S9-mix was carried out at a range of 10 concentrations from 6.2 to 961 μg/mL (approximately 10 mM). No cytotoxicity was observed. The concentrations used in this experiment were considered appropriate and therefore the data could be used for micronuclei evaluation (i.e. Experiment 1).

In experiment 2, six concentrations ranging from 58.6 to 961 μg/mL were applied in the 28 h treatment in the absence of S9-mix.

No precipitation of the test item was noted in any of the exposure conditions.

Positive controls were cyclophosphamide, mitomycin C and vinblastine. Deionised water was used as vehicle control.

CBPI cytotoxicity data were used to select the concentrations for the MN analysis. For all three exposure scenarios, the following concentrations were chosen for the MN analysis: 314, 549 and 961 μg/mL.

In the treatment of 3 h + 25 h in the absence of S9-mix, a cytotoxicity of 2.5% and 2.9% was reported only at 314 and 549 μg/mL, respectively.

In the treatment of 3 h + 25 h in the presence of S9-mix, a cytotoxicity of 4.6% and 8.3% was reported only at 314 and 549 μg/mL, respectively.

In the treatment of 28 h in the absence of S9-mix, the concentrations of 314, 549 and 961 μg/mL produced cytotoxicity of 9.3%, 4.3% and 0.6%, respectively.

In all three test conditions, 4H-pyran-4-one did not induce statistically significant increases in the frequency of micronucleated cells compared to vehicle controls nor concentration-dependent trends.

Therefore, the Panel concluded that 4H-pyran-4-one did not induce micronuclei in human peripheral blood lymphocytes in this study. The Panel considered the study to be reliable without restrictions and its result of high relevance.

Results of these *in vitro* studies on 4H-pyran-4-one are summarised in Appendix D, Table D.1.

Conclusion: Given the positive results of the bacterial gene mutation assay, an *in vivo* follow-up study would be needed because the exposure to 4H-pyran-4-one exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 18). The Panel acknowledges the submission of a follow-up *in vivo* Comet assay for this component (Documentation provided to EFSA N. 6). However, because this spontaneous submission[20] happened too late during the risk assessment process (i.e. on 22 September 2023), this additional study could not be evaluated by the Panel in the present opinion. The Panel noted that the results would not have an impact on the overall conclusions on the Primary Product, since furan-2(5*H*)-one and benzene-1,2-diol, two known *in vivo* genotoxic substances via the oral route, are present in the Primary Product resulting in an exposure that exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Section 5).

B.8. 2,5-Hexanedione (CAS No. 110-13-4)

Four papers on 2,5-hexanedione, retrieved from the literature, were submitted by the applicant.

Aeschbacher et al. (1989) tested the substance in a group of about 40 coffee aroma constituents in a bacterial reverse mutation assay on three *S*. Typhimurium (TA98, TA100 and TA102) with and without metabolic activation, with negative results. The test conduct was overall in line with the current standard, however the set of the bacterial strains used was not complete compared to the recommendations of OECD TG 471 (OECD, 2020b). The study is of limited reliability and relevance.

[20] For spontaneous submission please refer to the EFSA 'Administrative guidance for the processing of applications for regulated products (update 2021)' (EFSA, 2021), see section 2.13 'Spontaneous submission of information during the life-cycle of an application'.

Zimmermann et al. (1989) and Mayer and Goin (1994) tested the substance for the induction of mitotic chromosome loss in the D61.M strain of the yeast *Saccharomyces cerevisiae*, an obsolete assay not validated for regulatory purposes. The first study found the 2,5-hexanedione weakly positive, with an apparent synergic effect with propionitrile. The second study reported a clearly positive outcome. These two studies indicate a possible aneugenic effect. Both studies are of limited reliability and relevance.

Muhammad et al., 2018 reported induction of 'DNA fragmentation in the blood' micronuclei in the bone marrow of rats after oral administration. The technical procedure used to investigate DNA fragmentation is not clearly described. In addition, this endpoint could reflect cytotoxic effect rather than genotoxicity. The micronucleus assay is considered inconclusive, because of several limitations in the description of the experimental procedure and in data reporting. The study is of limited reliability and relevance.

Overall, the data set for 2,5-hexanedione is incomplete: the Ames test was not conducted in the complete set of bacterial strains recommended by OECD and the other available studies are insufficient to conclude on the possible induction of structural and numerical chromosomal aberrations. Two non-guideline studies indicated a possible aneugenic activity. It should be noted that this indication is supported by mechanistic studies that reported formation of covalently crosslinked tubulin dimer in an acellular *in vitro* system (Boekelheide, 1987a) and alterations in microtubule assembly induced by the substance in experimental animals after oral exposure (Boekelheide, 1987b).

Conclusion: Given the limited relevance of the available data set and the indications for structural and numerical chromosomal aberrations, appropriate *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 2,5-hexanedione exceeds the TTC for DNA-reactive mutagens and/or carcinogens (see Table 18).

B.9. 5-Acetyldihydro-2(3*H*)-furanone (CAS No. 29393-32-6)

The applicant performed the following two *in vitro* genotoxicity studies on 5-acetyldihydro-2(3*H*)-furanone, which were assessed by the Panel as described below.

Bacterial reverse mutation assay

5-Acetyldihydro-2(3*H*)-furanone (batch: ZY 211 086; purity > 97%) was tested in a bacterial reverse mutation assay according to OECD TG 471 (OECD, 2020b) and GLP principles.

Four strains of *S*. Typhimurium (TA98, TA100, TA1535, TA1537) and *E. coli* WP2 uvrA were used both in the absence and in the presence of metabolic activation by phenobarbital/β-naphthoflavone-induced rat liver S9 fraction (S9-mix). Three separate experiments were conducted. In Experiments 1 and 1a, the plate incorporation method was used and in Experiment 2 the pre-incubation method was used (ICCR, 2022c). Experiment 1a tested strain TA 98 with S9-mix only, as in Experiment 1 the negative and solvent control results for this strain were outside the test laboratory's range of historical values and therefore the results for this strain were invalid.

Positive control chemicals and DMSO (as vehicle control) were evaluated concurrently. All tests were evaluated in triplicate plates.

5-Acetyldihydro-2(3*H*)-furanone was tested at eight concentrations ranging from 3 to 5,000 μg/plate in Experiments 1 and 1a and at six concentrations ranging from 33 to 5,000 μg/plate in Experiment 2. No precipitation was observed in any experiment.

In all experiments, plates incubated with the test item showed normal background growth for all strains with and without S9-mix. No toxic effects occurred for any test condition.

In all experiments, no increase in the mean number of revertant colonies was observed at any tested concentration in any tester strain in the absence or presence of metabolic activation and results remained within the ranges of the solvent and untreated controls. The only exceptions were the number of revertant colonies in WP2 uvrA strain treated with 100 and 1,000 μg/plate with S9-mix in Experiment 1, which slightly exceeded the maximum value of the laboratory's historical vehicle controls, but without reaching a two-fold increase.

The Panel concluded that 5-acetyldihydro-2(3*H*)-furanone did not induce gene mutations in bacteria in this study. The Panel considered this study to be reliable without restrictions and its result of high relevance.

In vitro mammalian cell micronucleus test

5-Acetyldihydro-2(3H)-furanone (batch: ZY 211 086; purity > 97%) was tested in an *in vitro* micronucleus assay with cytokinesis block protocol, in accordance with OECD TG 487 (OECD, 2016e) and GLP. Two separate experiments were carried out in duplicate in human peripheral blood lymphocytes: 3 h exposures with and without S9-mix (phenobarbital/β-naphthoflavone-induced rat liver S9 fraction) were applied in Experiment 1 and 28 h exposure without S9-mix was applied in Experiment 2.

Positive controls were cyclophosphamide, mitomycin C and vinblastine. Deionised water, standard as solvent, was also used as negative control (ICCR, 2022d). A range-finder experiment using 3 h exposures with and without S9-mix was carried out in a range of 10 concentrations, from 8.6 to 1,321 μg/mL (approximately 10 mM). The concentrations used in this experiment were considered appropriate and therefore the data could be used for micronuclei evaluation (i.e. Experiment 1).

In experiment 2, lymphocytes were treated with six concentrations from 80.5–1,321 μg/mL for 28 h in the absence of metabolic activation.

No precipitate of the test item was noted in any of the exposure conditions.

CBPI cytotoxicity data were used to select the concentrations for MN analysis. For all three exposure scenarios, the following concentrations were chosen for the MN analysis: 431, 755 and 1,321 μg/mL.

In the treatment of 3 h + 25 h in the absence of S9-mix, weak cytotoxicity (9.6%) was reported only at 431 μg/mL.

In the treatment of 3 h + 25 h in the presence of S9-mix, cytotoxicity of 15.3% and of 13.3% were reported only at 431 and 1,321 μg/mL, respectively.

In the treatment of 28 h in the absence of S9-mix, a cytotoxicity of 20.4%, 25.2% and 23.3% were reported at 431, 755 and 1,321 μg/mL, respectively.

Only in the 3 h exposure in the presence of S9-mix, at 755 μg/mL the mean percentage of micronucleated cells (1.0%) was statistically significantly increased, when compared with the solvent control (0.55%), and also slightly exceeded the 95% control limit of the historical solvent control (0.00–0.97% micronucleated cells). Since this value was well within the min-max range of the historical solvent control (0.05–1.15% micronucleated cells) and there was no concentration-related increase in MN formation, as evaluated by a trend test, this minor increase was judged by the study authors to be biologically irrelevant. In all other test conditions, there were no statistically significant increases compared to vehicle controls, no concentration-related trends and results were within the 95% control limits of the historical controls. Therefore, the Panel concluded that 5-acetyldihydro-2(3H)-furanone did not induce micronuclei in human peripheral blood lymphocytes in this study. The Panel considered the study to be reliable without restrictions and its result of high relevance.

Results of the *in vitro* studies on 5-acetyldihydro-2(3*H*)-furanone are summarised in Appendix D, Table D.1.

Conclusion: the data available for 5-acetyldihydro-2(3*H*)-furanone rule out the concern for genotoxicity of this component, that was identified by the applicant. Accordingly, no further risk assessment based on comparison of exposure with the TTC of 0.0025 μg/kg bw/day is needed.

B.10. Pyrrole-2-carbaldehyde former (CAS No. 1003-29-8, former [FL-no: 14.145])

The applicant identified in the literature an *in vitro* MN study in human peripheral blood lymphocytes reporting that pyrrole-2-carbaldehyde induced a statistically significant concentration-related increase in micronucleated cells (Skoutelis et al., 2017). The study was conducted in whole blood cultures in the absence of metabolic activation with test concentrations of 5, 10 and 25 μg/mL. The Panel considered this study as reliable with restrictions (due to limitations in the experimental protocol) and the results of limited relevance.

Based on these data, the applicant tested pyrrole-2-carbaldehyde in an *in vivo* combined comet and MN study (BSRC, 2023).

The applicant submitted a validated analytical method for the determination of pyrrole-2-carbaldehyde (CAS No. 1003-29-8) in 0.5% (w/v) methylcellulose using HPLC and demonstrated the stability of the substance (BSRC, 2022d,e) (Documentation provided to EFSA No. 3).

In vivo combined bone marrow micronucleus test and comet assay

The genotoxic potential of pyrrole-2-carbaldehyde (batch: XY2SJ, purity 100%) was assessed *in vivo* using the bone marrow micronucleus assay combined with the comet assay in the (glandular) stomach, duodenum and liver of Crl:CD(SD) [SPF] male rats (BSRC, 2023). The study was conducted in compliance with GLP and in accordance with OECD TG 474 (OECD, 2016c) and OECD TG 489 (OECD, 2016d) for the micronucleus assay and for the comet assay, respectively. This study also included a TK analysis to evaluate systemic exposure to pyrrole-2-carboxaldehyde in rats.

In a dose range finding study, groups of three male and three female Crl:CD(SD) rats per dose group were given three consecutive daily administrations by oral gavage (at 0, 24 and 45 h) of pyrrole-2-carbaldehyde in 0.5% methylcellulose, at 250, 500, 1,000 and 2,000 mg/kg bw per day. Post-dose effects were reported following the first administration for all groups, except the low dose male and female groups, and included decreases in locomotor activity, irregular respiration, prone position, lateral position, ptosis, lacrimation, hypothermia and chromaturia (brown urine). In the top-dose males, there were no deaths or moribund animals but at 1,000 mg/kg bw per day one male was found moribund after the third administration. In the top-dose female group, after the second administration, one animal died and another was judged moribund; in the 1,000 mg/kg bw per day group another animal also died after the second administration. Suppression in bw gains were not observed at the lower doses of 250 and 500 mg/kg bw per day. Based on this study, 750 mg/kg bw per day was considered the MTD and doses of 250, 500 and 750 mg/kg/day were selected for the combined study.

As no gender differences were observed in the dose range finding study, the main experiment was performed only in male rats. Groups of six male Crl:CD(SD) rats per dose group were given three consecutive daily administrations by oral gavage (at 0, 24 and 45 h) of pyrrole-2-carbaldehyde at 0 (0.5% methylcellulose), 125, 250, 500 and 750 mg/kg bw per day. A positive control group of six male rats was dosed via oral gavage for two consecutive days (Days 2 and 3; 21 h interval) with 200 mg/kg bw per day EMS. Animals were killed and sampled at 3 h following the final administration. As there were no deaths or moribund animals observed at any dose, comet and micronuclei assessments were performed for the top three dose levels (250, 500 and 750 mg/kg bw per day) and five animals per group were evaluated. Following treatment with pyrrole-2-carbaldehyde, no clinical signs of toxicity were observed in animals of the 125 mg/kg bw per day group but at 250 mg/kg bw per day a decrease in locomotor activity was observed in two rats and ptosis was observed in one rat, at 500 mg/kg bw per day decreases in locomotor activity and chromaturia (brown) were observed in all rats, and at 750 mg/kg bw per day a decrease in locomotor activity, irregular respiration and chromaturia (brown) were observed in all rats, lacrimation was observed in four rats and prone position was observed in one rat.

There was a dose-related decrease in bw gains (approximately 10% at the top dose). There were no gross necropsy findings, upon macroscopic examination, related to pyrrole-2-carboxaldehyde treatment in the liver, stomach or duodenum.

To demonstrate bone marrow exposure of Crl:CD(SD) rats treated with pyrrole-2-carbaldehyde, TK analyses for satellite groups were performed. A method using LC–MS/MS was developed for the determination and subsequent analysis of pyrrole-2-carbaldehyde and, its metabolite, pyrrole-2-carboxylic acid in rat plasma (BSRC, 2022f). In the validation study for this method, the range of 10–10,000 ng/mL for both pyrrole-2-carbaldehyde and pyrrole-2-carboxylic acid in rat plasma was used. The study also confirmed the validity of the method for samples of pyrrole-2-carbaldehyde and pyrrole-2-carboxylic acid in rat plasma that had been stored for three consecutive days in a refrigerator (acceptable temperature range: 1–9°C). In contrast, samples that had undergone freeze–thaw or had been frozen for 4 weeks, took on a gel state and were not suitable for analyses.

Groups of four male Crl:CD(SD) rats per dose group were treated with the same doses as per the main study, administered as a single administration on Day 2. Blood was collected from the jugular vein at 1, 2, 4 and 8 h after administration. Samples from the vehicle control, 250 and 750 mg/kg bw per day groups (three animals per group) were selected for the TK analysis.

Comet assay

Stomach, liver and duodenum cells were prepared for comet analysis. Median tail DNA % values for a total of 150 cells per organ per animal (five animals in total) were recorded. No dose-related increase in hedgehogs was observed following treatment with pyrrole-2-carbaldehyde for any tissue.

In stomach, liver and duodenum, no statistically significant increases in group mean (of median) tail DNA % values were observed in any pyrrole-2-carbaldehyde treatment group compared to the vehicle control group and the frequencies of % tail DNA were within the respective ranges of the historical negative control data for the test laboratory. Furthermore, tail DNA % for the positive control groups were within the acceptable ranges of the respective historical positive control data at the test laboratory and increased with statistically significant differences compared to the respective concurrent negative control. As no increases in DNA damage were observed in the liver, (glandular) stomach and duodenum, histopathological examinations were not conducted.

Micronucleus assay

Bone marrow from the femurs was prepared for micronucleus scoring. A total of 500 erythrocytes were counted to determine the number of immature erythrocytes as a marker of the effect of pyrrole-2-carbaldehyde on bone marrow cell proliferation. For MN analysis, 4,000 immature erythrocytes per animal (five animals per group) were scored for the presence of MN.

All groups treated with pyrrole-2-carbaldehyde exhibited group mean frequencies of micronucleated immature erythrocytes (MNIE) that were not statistically different from those observed in the concurrent vehicle control. Furthermore, the mean frequency MNIE for the positive control group was within the acceptable range of the historical positive control data at the test laboratory and increased with a statistically significant difference compared to the negative control. Compared to the vehicle control, treatment with pyrrole-2-carbaldehyde did not statistically significantly decrease the ratio of immature to total erythrocytes, and therefore no indication of a notable effect on bone marrow cell proliferation was observed.

TK analysis

In the TK analysis, the tmax, Cmax and AUC_{0-8h} of pyrrole-2-carbaldehyde for the 750 mg/kg bw per day group were 1.0 h, 42 μg/mL and 130 μg·h/mL, respectively. At the same dose, the tmax, Cmax and AUC_{0-8h} of pyrrole-2-carboxylic acid were 2.7 h, 163 μg/mL and 1,000 μg·h/mL, respectively. At the lower dose of 250 mg/kg bw per day group, the tmax, Cmax and AUC_{0-8h} were respectively 2.0 h, 15 μg/mL and 29 μg·h/mL for pyrrole-2-carbaldehyde and 4.0 h, 96 μg/mL and 519 μg·h/mL for pyrrole-2-carboxylic acid. Therefore, systemic exposure to pyrrole-2-carbaldehyde in rats was confirmed.

The Panel considered this study as reliable without restrictions and the results of high relevance. Thus, it was concluded that pyrrole-2-carbaldehyde did not induce DNA damage in the rat liver, (glandular) stomach and duodenum *in vivo*, nor did it increase the incidence of micronucleated erythrocytes in rat bone marrow cells *in vivo* when tested up to the MTD in this study in which systemic exposure to the test substance and its major metabolite was confirmed.

Study results are summarised in Appendix D, Table D.2.

Conclusion: the data available for pyrrole-2-carbaldehyde rule out the concern for genotoxicity of this component that was identified by the applicant. Accordingly, no further risk assessment based on comparison of exposure with the TTC of 0.0025 μg/kg bw/day is needed.

B.11. 1-Acetyl-1-cyclohexene (CAS No. 932-66-1)

The applicant performed the following two *in vitro* genotoxicity studies on 1-acetyl-1-cyclohexene, which were assessed by the Panel as described below.

Bacterial reverse mutation assay

A bacterial reverse mutation assay was conducted in *S*. Typhimurium strains TA98, TA100, TA1535, TA1537 and in *E. coli* WP2 uvrA to assess the mutagenicity of 1-acteyl-1-cyclohexane (batch: ZY_212_144_Pr4; purity ≥ 96.5%), both in the absence and in the presence of metabolic activation by phenobarbital/β-naphthoflavone-induced rat liver S9 fraction (S9-mix). Three separate experiments were conducted. In Experiment 1, the plate incorporation method was used and in Experiments 2 and 2a the pre-incubation method was used (ICCR, 2022g). Experiment 2a tested strain TA 98 without S9-mix only and was conducted to confirm the findings from the Experiment 2. Study design complied with OECD TG 471 (OECD, 2020b) and the GLP principles.

Positive control chemicals and DMSO (as vehicle control) were evaluated concurrently. All tests were evaluated in triplicate plates.

1-Acteyl-1-cyclohexane was tested at eight concentrations ranging from 3 to 5,000 μg/plate in Experiment 1 and at nine concentrations ranging from 10 μg/plate (strain TA98 without S9-mix) or at six concentrations ranging from 33 μg/plate (strain TA98 with S9-mix and all other strains with or without S9-mix) to 5,000 μg/plate in Experiment 2. In Experiment 2a, strain TA98 without S9-mix was tested at seven concentrations ranging from 100 to 2,000 μg/plate. No precipitation was observed in any experiment.

In Experiments 1 and 2, plates incubated with the test item showed reduced background growth for all strains with and without S9-mix at the highest concentration tested. Toxic effects only occurred in Experiment 1 for WP2 uvrA in the presence of S9-mix, in Experiment 2 for all strains in the presence and absence of S9-mix except for WP2 uvrA in the absence of S9-mix and did not occur in Experiment 2a.

In Experiment 1, for strain TA98 without S9-mix minor increases (not reaching the two-fold threshold) in the mean number of revertant colonies was observed at 1,000 μg/plate and 2,500 μg/plate that also exceeded the maximum value of the historical vehicle controls. This effect for strain TA98 without S9-mix was nearly reaching a two-fold increase at 333 μg/plate and also the maximum value of the historical vehicle controls in Experiment 2, but it was not repeated in Experiment 2a.

Therefore, the Panel considered that the increase in Experiment 2 was not reproducible and concluded that 1-acetyl-1-cyclohexene did not induce gene mutations in bacteria in this study. The Panel considered the study to be reliable without restrictions and its result of high relevance.

In vitro mammalian cell micronucleus test

1-Acteyl-1-cyclohexane (batch: ZY_212_144_Pr4; purity ≥ 96.5%) was tested in an *in vitro* micronucleus assay, cytokinesis block protocol, in accordance with OECD TG 487 (OECD, 2016e) and GLP. Three separate experiments were performed in duplicates in human peripheral blood lymphocytes: 3 h exposures with and without metabolic activation by phenobarbital/β-naphthoflavone-induced rat liver S9 fraction (S9-mix) were conducted in Experiment 1, a 28 h exposure without S9-mix was conducted in Experiment 2 and an additional 3 h exposure without S9-mix was conducted as a confirmatory test in Experiment 3. Positive controls were cyclophosphamide, mitomycin C and vinblastine. DMSO was used as vehicle control (ICCR, 2022h).

Ten concentrations of 1-acteyl-1-cyclohexane, ranging from 4.9 to 1,287 μg/mL, were tested in the range-finder experiment using 3 h exposures with and without S9-mix. At the highest concentration of 1,287 μg/mL (approximately 10 mM) phase separation was observed at the end of 3 h treatments both with and without S9-mix (Experiment 1 only). The concentrations used in this experiment were considered appropriate and therefore the data could be used for micronuclei evaluation (i.e. Experiment 1).

For experiment 2 (28 h treatment in the absence of S9-mix), lymphocytes were treated with 10 concentrations of 1-acteyl-1-cyclohexane ranging from 37.0 to 1,287 μg/mL. In Experiment 3 (3 h treatment in the absence of S9-mix), seven concentrations ranging from 44.8 to 1,287 μg/mL were tested.

CBPI cytotoxicity data were used to select the concentrations for the MN analysis.

In the treatment of 3 h + 25 h in the absence of S9-mix (Experiment 1), the following concentrations were chosen for MN analysis: 245, 429 and 1,287 μg/mL (cytotoxicity of 10.9%, 15.4% and 35.3%, respectively). The frequency of micronucleated cells at the top concentration (1.70%) was statistically significantly increased compared to the vehicle control (0.60%) and increases in micronuclei frequency were concentration-related (trend test: $p < 0.05$). Furthermore, the micronuclei frequency at the top concentration exceeded the 95% control limits (0.01–0.92%) and the min-max range (0.15–1.15%) of the historical vehicle controls.

In Experiment 3, using the same conditions (i.e. 3 h + 25 h in the absence of S9-mix), the following concentrations were chosen for MN analysis: 420, 735 and 1,287 μg/mL (cytotoxicity of 22.0%, 25.0% and 35.3%, respectively). None of the findings from the first experiment were observed and the study authors concluded that the findings in the first experiment were biologically irrelevant.

In the treatment of 3 h + 25 h in the presence of S9-mix (Experiment 1), the following concentrations were chosen for MN analysis: 245, 429 and 1,287 μg/mL (cytotoxicity was not detected at any concentration).

In the treatment of 28 h in the absence of S9-mix (Experiment 2), the following concentrations were chosen for MN analysis: 64.7, 113 and 198 μg/mL (cytotoxicity of 26.9%, 25.5% and 40.6%, respectively).

In these two other test conditions (3 h + 25 h in the presence of S9-mix and 28 h continuous exposure in the absence of S9-mix), 1-acteyl-1-cyclohexane did not result in statistically significant increases in the frequency of micronucleated cells compared to vehicle controls nor concentration-dependent trends.

The Panel concluded that 1-acteyl-1-cyclohexane did not induce micronuclei in human peripheral blood lymphocytes in this study. The Panel considered the study to be reliable without restrictions and its result of high relevance.

Results of these *in vitro* studies on 1-acteyl-1-cyclohexane are summarised in Appendix D, Table D.1.

Conclusion: the data available for 1-acteyl-1-cyclohexane rule out the concern for genotoxicity of this component, that was identified by the applicant. Accordingly, no further risk assessment based on comparison of exposure with the TTC of 0.0025 μg/kg bw/day is needed.

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

(4) Reliability cannot be evaluated[22] '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:

[21] Klimisch et al. (1997) used the term 'Not reliable' however, 'Reliability Insufficient' was considered more appropriate.

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

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

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

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

Appendix D – Genotoxicity studies on individual components

Table D.1: Summary of *in vitro* genotoxicity studies on individual components of Smoke Concentrate 809045 (SF-003)

Chemical name CAS No.	Test system *in vitro*	Test object	Concentrations[(a)] and test conditions	Result	Reliability/ comments	Relevance of test system/ relevance of the result	Reference
1-Acteyl-1-cyclohexane 932-66-1	Reverse Mutation test	*S.* Typhimurium TA98, TA100, TA1535, TA1537 *E. Coli* WP2 uvrA	Experiment 1 (plate incorporation): 3–5,000 μg/plate (+S9, −S9) Experiment 2 (pre-incubation): 10–5,000 μg/plate (TA 98 −S9); 33–5,000 μg/plate (TA 98 + S9 and the remaining strains −S9, +S9) Experiment 2a (pre-incubation): 100–2,000 μg/plate (TA 98 −S9)	Negative	Reliable without restrictions. Study performed according to OECD TG 471 and in compliance with GLP.	High/high	ICCR (2022g)
	Micronucleus assay	Human peripheral blood lymphocytes	Experiment 1: 245, 429 and 1287 μg/mL (3 + 25 h, +S9, −S9) Experiment 2: 64.7, 113 and 198 μg/mL (28 h, −S9) Experiment 3: 420, 735 and 1287 μg/mL (3 + 25 h, −S9)	Negative	Reliable without restrictions.Study performed according to OECD TG 487 and in compliance with GLP.	High /High	ICCR (2022h)
4H-pyran-4-one 108-97-4	Bacterial Reverse Mutation test	*S.* Typhimurium TA98, TA100, TA1535, TA1537 *E. Coli* WP2 uvrA	Experiment 1 (plate incorporation): 3–5,000 μg/plate (+S9, −S9) Experiment 2 (pre-incubation): 33–5,000 μg/plate (+S9, −S9) Experiment 2a (pre-incubation): 33–5,000 μg/plate (+S9)	Positive	Reliable without restrictions. Study performed according to OECD TG 471 and in compliance with GLP.	High/High	ICCR (2022e)
	Micronucleus assay	Human peripheral blood lymphocytes	Experiment 1: 314, 549 and 961 μg/mL (3 + 25 h, −S9) 314, 549 and 961 μg/mL (3 + 25 h, +S9)	Negative	Reliable without restrictions. Study performed according to OECD TG	High /high	ICCR (2022f)

Chemical name CAS No.	Test system *in vitro*	Test object	Concentrations[(a)] and test conditions	Result	Reliability/ comments	Relevance of test system/ relevance of the result	Reference
			Experiment 2: 314, 549 and 961 μg/mL (28 h, −S9)		487 and in compliance with GLP.		
5-Acetyldihydro-2(3H)-furanone 29393-32-6	Bacterial Reverse Mutation test	*S.* Typhimurium TA98, TA100, TA1535, TA1537 *E. Coli* WP2 uvrA	Experiment 1 (plate incorporation): 3–5,000 μg/plate (+S9, −S9) Experiment 1a (plate incorporation): 3–5,000 μg/plate (TA 98 only +S9, −S9) Experiment 2 (pre-incubation): 33–5,000 μg/plate (+S9, −S9)	Negative	Reliable without restrictions. Study performed according to OECD TG 471 and in compliance with GLP.	High/high	ICCR (2022c)
	Micronucleus assay	Human peripheral blood lymphocytes	Experiment 1: 431, 755 and 1,321 μg/mL (3 + 25 h, −S9) 431, 755 and 1,321 μg/mL (3 + 25 h, +S9) Experiment 2: 431, 755 and 1,321 μg/mL (28 h, −S9)	Negative	Reliable without restrictions. Study performed according to OECD TG 487 and in compliance with GLP.	High/high	ICCR (2022d)
3-Methoxycatechol 934-00-9	Bacterial Reverse Mutation test	*S.* Typhimurium TA98, TA100, TA1535, TA1537 *E. Coli* WP2 uvrA	Experiment 1 (plate incorporation): 3–5,000 μg/plate (+/−S9) Experiment 2 (pre-incubation): 3–2,500 μg/plate (−S9); 33–5,000 μg/plate (TA 100, +S9); 10–5,000 μg/plate (remaining strains +S9) Experiments 1a (plate incorporation) and 2a (pre-incubation): 33–5,000 (TA 100, +S9)	Equivocal (weak response in TA100 + S9-mix)	Reliable without restrictions. Study performed according to OECD TG 471 and in compliance with GLP.	High/Limited	ICCR (2022a)
	Micronucleus assay	Human peripheral blood lymphocytes	Experiment 1: 28.2, 49.4, 86.5 μg/mL (3 + 25 h, −S9)	Negative	Reliable without restrictions.	High/high	ICCR (2022b)

Chemical name CAS No.	Test system *in vitro*	Test object	Concentrations[(a)] and test conditions	Result	Reliability/ comments	Relevance of test system/ relevance of the result	Reference
			16.1, 49.4, 151 μg/mL (3 + 25 h, +S9) Experiment 2: 17.6, 30.8, 70 μg/mL (28 h, −S9)		Study performed according to OECD TG 487 and in compliance with GLP.		

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

Table D.2: Summary of *in vivo* genotoxicity studies on individual components of Smoke Concentrate 809045 (SF-003)

Chemical name CAS No.	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
Furan-2(5*H*)-one 497-23-4	Micronucleus assay in liver	Crl:CD(SD) rats; M gavage	50, 100 and 200[(a)]	Positive	Reliable without restrictions. Study performed in compliance with GLP. An OECD test guideline is not yet available, however, the study was performed following a validated protocol recommended by the international workshops on genotoxicity testing (IWGT) (Hamada et al., 2015; Uno et al., 2015; Kirkland et al., 2019).	High/high	LSIM (2022a)
Pyrrole-2-carbaldehyde 1003-29-8	Micronucleus assay in bone marrow	Crl:CD(SD) rats; M gavage	125, 250, 500, 750[(b)]	Negative	Reliable without restrictions. Study performed according to OECD TG 474 and in compliance with GLP. The highest dose tested (750 mg/kg bw per day) was an estimate of the MTD according to the dose range finding study.	High/high	BSRC (2023)
	Comet assay in liver, stomach and duodenum			Negative	Reliable without restrictions. Study performed according to OECD TG 489 and in compliance with GLP.	High/high	BSRC (2023)

bw: body weight; M: males.
(a): The test substance was administered once daily for 28 consecutive days; sampling 24 h after the final administration.
(b): The test substance was administered once daily on three consecutive days; sampling 3 h after the final administration.

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

Table E.1: Summary of *in vitro* genotoxicity studies on Smoke Concentrate 809045 (SF-003) 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
Smoke Concentrate 809045	Bacterial Reverse Mutation test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	50–5,000 μg/plate (+/−S9, plate incorporation)	Inconclusive (Equivocal for the strains used)	Reliable with restrictions (incomplete battery of bacterial strains: *S. typhimurium* TA102/ *E. coli* WP2 uvrA lacking) Study performed according to OECD TG 471 and in compliance with GLP	High/low	Freiburger Labor für Mutagenitätsprüfung (1998a)
	In vitro mammalian cell gene mutation test in mouse lymphoma cells	L5178Y $TK^{+/-}$ mouse lymphoma cells	Experiment 1: 0.19–290 μg/mL (24 h, −S9) 0.77–600 μg/mL (4 h, +S9) Experiment 2: 0.65–98 μg/mL (24 h −S9) 1.3–300 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 (2005)
	In vitro mammalian chromosomal aberrations test	Chinese hamster ovary cells (CHO K-1 cell line)	Experiment 1: 100, 125, 250 μg/mL (4 + 18 h, +S9) 25, 50, 125 μg/mL (4 + 18 h, −S9) Experiment 2: 150, 200, 300 μg/mL (4 + 18 h, +S9) 50, 75, 100 μg/mL (4 + 18 h, −S9) 30, 75, 100 μg/mL (18 + 18 h, −S9)	Positive	Reliable with restrictions (200 metaphases per concentration instead of 300 were scored). Study performed according to OECD TG 473 and in compliance with GLP	High/limited	TNO (2004)

Table E.2: Summary of *in vivo* genotoxicity studies on Smoke Concentrate 809045 (SF-003) 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
Smoke Concentrate 809045	Micronucleus assay in bone marrow	NMRI BR mice; M and F Oral	2,000[(a)]	Negative	Reliable with restrictions (only 1,000 cells counted). Study performed according to OECD TG 474 and in compliance with GLP.	High/limited	Freiburger Labor für Mutagenitätsprüfung (1998b)
	UDS assay in liver	Wistar rats; M Oral	2,000[(b)]	Negative	Reliable without restrictions. Study performed according to OECD TG 486 and in compliance with GLP and	Low/low	TNO (2007)

bw: body weight; M: males; F: females.
(a): One administration with sampling at: 24, 48 and 72 h.
(b): One administration with sampling at: 2–4 h and 12–16 h.

Appendix F – New genotoxicity study on the Primary Product (whole mixture)

Table F.1: Summary of *in vivo* genotoxicity study on Smoke Concentrate 809045 (SF-003)

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
Smoke Concentrate 809045	Gene mutation assay in liver, stomach and duodenum	Muta™Mouse (lacZ/GalE) CD2-LacZ80/HazfBR SPF transgenic mice; M gavage	250, 500 and 1,000	Negative	Reliable with restrictions (higher doses could have been applied). Study performed according to OECD TG 488 and in compliance with GLP	High/limited	BSRC (2022a)

bw: body weight; M: males.

Annex A – Exposure assessment results

- Annex A1 Occurrence data per food category considered in FAIM, (mg/kg).
- Annex A2: Total estimated exposure of Smoke Concentrate 809045 (SF-003) 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 Smoke Concentrate 809045 (SF-003) 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 Smoke Concentrate 809045 (SF-003) 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 Smoke Concentrate 809045 (SF-003) 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 Smoke Concentrate 809045 (SF-003) 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 Smoke Concentrate 809045 (SF-003) 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.