Adopted: 18 September 2024

DOI: 10.2903/j.efsa.2024.9030

efsa JOURNAL

SCIENTIFIC OPINION

Assessment of the feed additive consisting of a preparation containing a smoke flavouring extract for cats and dogs for the renewal of the authorisation (Azelis Denmark A/S)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) | **Roberto Edoardo Villa** | **Giovanna Azimonti** | **Eleftherios Bonos** | **Henrik Christensen** | **Mojca Durjava** | **Birgit Dusemund** | **Ronette Gehring** | **Boet Glandorf** | **Maryline Kouba** | **Marta López-Alonso** | **Francesca Marcon** | **Carlo Nebbia** | **Alena Pechová** | **Miguel Prieto-Maradona** | **Ilen Röhe** | **Katerina Theodoridou** | **Maria de Lourdes Bastos** | **Paul Brantom** | **Andrew Chesson** | **Josef Schlatter** | **Johannes Westendorf** | **Paola Manini**

Correspondence: feedap@efsa.europa.eu

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the assessment of the application for the renewal of authorisation of a preparation containing a smoke flavouring extract for cats and dogs. The applicant provided data demonstrating that the additive currently on the market does not fully comply with the conditions of authorisation, but with newly proposed specifications based on different analytical methods. Considering that the additive under assessment contains benzofuran and styrene, for which a potential concern for genotoxicity has been identified, and that the whole mixture raises a potential concern for genotoxicity, additional data would be needed to complete the assessment. Therefore, the FEEDAP Panel is not in the position to conclude on the safety for cats and dogs. The additive is authorised for use in feed for cats and dogs, and therefore, there is no need to perform an assessment of the safety for the consumer and the environment. Regarding user safety, the additive should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. When handling the additive, exposure of unprotected users to potential genotoxic substances may occur. Therefore, to reduce the risk, the exposure of the users should be minimised. There was no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

KEYWORDS

benzofuran, efficacy, renewal, safety, sensory additives, smoke flavouring extract, styrene

CONTENTS

Abstract 1
1. Introduction 3
1.1. Background and Terms of Reference 3
1.2. Additional information 3
2. Data and Methodologies 3
2.1. Data 3
2.2. Methodologies 4
3. Assessment 4
3.1. Characterisation 4
3.1.1. Characterisation of the additive 4
3.1.1.1. Characterisation of the volatile fraction 4
3.1.1.2. Characterisation of the non-volatile fraction 6
3.1.1.3. Impurities 6
3.1.2. Physical properties of the additive 6
3.1.3. Stability 7
3.1.4. Conditions of use 7
3.2. Safety 7
3.2.1. Genotoxicity 7
3.2.1.1. Analysis of the genotoxic activity of the individual identified components 8
3.2.1.2. Genotoxicity assessment of the additive (whole mixture) 8
3.2.1.3. Overall conclusions on genotoxicity 9
3.2.2. Safety for the target species 9
3.2.2.1. Benzofuran and styrene 9
3.2.2.2. Additive under assessment (whole mixture) 9
3.2.2.3. Polycyclic aromatic hydrocarbons 9
3.2.2.4. Conclusions on safety for the target species 10
3.2.3. Safety for the user 10
3.3. Efficacy 10
4. Conclusions 10
Abbreviations 11
Acknowledgements 11
Conflict of interest 11
Requestor 11
Question number 11
Copyright for non-EFSA content 11
Panel members 11
References 11

1 | INTRODUCTION

1.1 | Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest 1 year before the expiry date of the authorisation.

The European Commission received a request from Azelis Denmark A/S[2] for the renewal of the authorisation of the additive consisting of a preparation containing a smoke flavouring extract-2b0001 (Scansmoke PET SEF 7525), when used as a feed additive for cats and dogs (category: sensory additives; functional group: flavourings).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 14(1) (renewal of the authorisation). The dossier was received on 20 December 2023 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00900. The particulars and documents in support of the application were considered valid by EFSA as of 12 March 2024.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of a preparation containing a smoke flavouring extract, when used under the proposed conditions of use (see Section 3.1.4).

1.2 | Additional information

The additive is a preparation containing a smoke flavouring extract. The additive is currently authorised for use in feed for cats and dogs (2b0001).[3] EFSA issued an opinion on the safety and efficacy of this product when used in feed for cats and dogs (EFSA FEEDAP Panel, 2012).

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of a preparation containing a smoke flavouring extract as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 18 March 2024 to 18 June 2024; the comments received were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] 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 EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[6] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 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 technical dossier from 23 May to 13 June 2024 for which no comments were received.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substance in the feed additive are valid and applicable for the current application.[7]

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2]Azelis Denmark A/S, Lyskaer 5 Herlev, Denmark.
[3]COMMISSION IMPLEMENTING REGULATION (EU) No 1076/2014 of 13 October 2014 concerning the authorisation of a preparation containing a smoke flavouring extract-2b0001 as feed additive for dogs and cats. OJ L 269, 14.10.2014, p. 19.
[4]Dossier reference: FEED-2023-19350.
[5]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.
[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.
[7]Evaluation report available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en.

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of a preparation containing a smoke flavourings extract is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021a), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019a), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019b), Scientific Opinion on the guidance on aneugenicity assessment (EFSA Scientific Committee, 2021) and General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021b).[9]

3 | ASSESSMENT

The additive consisting of a preparation containing a smoke flavouring extract is currently authorised as sensory additive (functional group: flavouring compounds) for feed for cats and dogs. The assessment regards the renewal of the authorisation. Other names used in the current and previous assessments (Scansmoke SEF7525, smoke flavour Primary Product – Scansmoke SEF7525) refer to the additive under assessment.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The smoke flavouring extract, liquid form, is produced by diethyl ether extraction from tar produced by pyrolysis of a ratio of the following woods: 35% red oak (*Quercus rubra*), 35% white oak (*Quercus alba*), 10% maple (*Acer saccharum*), 10% beech (*Fagus grandifolia*) and 10% hickory (*Carya ovata*).

The applicant declared that the manufacturing process has not been changed since the previous authorisation.

The additive, as currently authorised, is specified to contain water 0.3–0.9 weight (wt) %, total acids (expressed as acetic acid) 0.06–0.25 meq/g, carbonyl compounds 1.2–3.0 wt %, phenols 8.0–12.0 wt % and to have a pH of 1–4.

The applicant is proposing to change the specifications for carbonyl compounds to 4.0–6.0 wt %, total acids (expressed as acetic acid) to 0.43–1.64 wt % (corresponding to 0.07–0.27 meq/g) and phenols (expressed as syringol) to 7.0–12.0 wt %. According to the applicant, this difference in the concentration range of carbonyl compounds is not due to changes in the manufacturing process but is related to differences in the performance of the analyses that are based on the reaction of carbonyls with hydroxylamine. However, analytical data to support this statement were not provided. The FEEDAP Panel notes that the method of analysis used to check specifications is different from the one used previously and has not been evaluated by the EURL.

The analysis of six batches of the additive[10] showed compliance with the new proposed specifications: total acids 1.00% (range: 0.49–1.5 wt %, corresponding to 0.08–0.25 mEq/g), carbonyl compounds 4.75% (4.4–5.4 wt %), phenols 8.8% (8.1–9.9 wt %), water 0.43% (0.30–0.62 wt %).

3.1.1.1 | *Characterisation of the volatile fraction*

The volatile fraction accounts on average for 40.1 wt % (33.4–45 wt %) of the additive. Forty-one compounds were identified and quantified in the volatile fraction[11] by applying a validated method based on gas chromatography–mass spectrometry (GC–MS).[12] The identified compounds accounted for 80.9 wt % (79.1–82.2 wt %) of the volatile fraction and for 32.4 wt % (27–37 wt %) of the additive. The unidentified volatile compounds accounted for 7.4 wt % (5.9–8.3 wt %) of the additive. The analysis of the volatile compounds in the six batches[13] is presented in Table 1. For the compounds which are listed in the authorising Regulation (EU) No 1076/2014, a comparison with the ranges reported in the authorisation for the feed additive is also presented.

[8]Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[9]https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf.

[10]Annex_01_Chemical characterisation_AR_AM.

[11]Annex_06_VOC_AR.

[12]Annex_07_Validation_AM_VOC.

[13]Annex_12_Batch_to_batch_AR.

TABLE 1 Constituents of the volatile fraction of the smoke flavouring extract: Batch to batch variation based on the analysis of six batches by gas chromatography–mass spectrometry (GC–MS).

Compound	CAS No	FLAVIS No	Range as reported in Regulation (EU) No 1076/2014	Mean wt %	Range wt %
Compounds listed in Regulation 1076/2014					
Syringol (2,6-dimethoxyphenol)	91-10-1	04.036	12.6–25.2	4.94	4.05–6.68
4-Methyl syringol (2,6-dimethoxy-4-methylphenol)	6638-05-7	04.053	6.2–9.2	3.42	2.55–4.04
4-Propenyl syringol (*trans*) (2,6-dimethoxy-4-(2-propenyl)phenol, *E*-)	20675-95-0	04.055	0.8–3.6	1.44	1.04–2.05
4-Ethyl syringol (4-ethyl-2,6-dimethoxyphenol)	14059-92-8	04.052	2.7–3.1	2.21	1.61–2.55
4-Methyl guaiacol (creosol/methylguaiacol)	93-51-6	04.007	2.0–2.6	2.28	1.94–2.54
4-Allyl syringol (2,6-dimethoxy-4-(2-propenyl)phenol)	6627-99-9	04.051	1.8–2.3	0.88	0.61–1.06
4-Ethyl guaiacol (4-ethyl-2-methoxyphenol)	2785-89-9	04.008	1.8–2.4	1.21	1.04–1.31
4-Propyl syringol (2,6-dimethoxy-4-propylphenol)	6766-82-1	04.056	1–2.5	0.11	0.09–0.13
Phenol, 2-methoxy- (guaiacol)	90-05-1	04.005	1.1–1.6	2.32	1.99–3.09
2,4-Dimethylphenol	105-67-9	04.066	0.9–1.4	1.28	1.09–1.37
Eugenol	97-53-0	04.003	1.0–1.4	0.64	0.53–0.71
Isoeugenol (*trans*)	5932-68-3	04.004	0.9–1.3	0.90	0.64–1.15
4-Propenyl syringol, cis (4-propenyl-2,6-dimethoxyphenol, *Z*-)	26624-13-5	04.055	0.3–1.7	0.59	0.43–0.68
o-Cresol (2-methyl phenol)	95-48-7	04.027	0.7–1.5	1.06	0.83–1.20
Phenol	108-95-2	04.041	0.5–1.2	0.93	0.76–1.04
p-Cresol (4-methyl phenol)	106-44-5	04.028	0.7–1.1	–	–
4-Propyl guaiacol (2-methoxy-4-propylphenol)	2785-87-7	04.049	0.5–1	0.46	0.43–0.50
Additional compounds					
2,6-Dimethylphenol	576-26-1	04.042		0.62	0.60–0.64
2,3-Dimethyl-2-cyclopenten-1-one	1121-05-7	–		0.58	0.51–0.61
3-Methylphenol	108-39-4	04.026		0.58	0.43–0.63
2,4,6-Trimethyl phenol	257-60-6	04.095		0.55	0.52–0.57
2-Furanmethanol (furfuryl alcohol)	98-00-0	13.019		0.39	0.33–0.46
4-Ethylphenol	123-07-9	04.022		0.35	0.28–0.44
3-Methyl-2-cyclopenten-1-one	2758-18-1	07.112		0.32	0.28–0.34
Acetic acid	64-19-7	08.002		0.30	0.29–0.31
Isoeugenol (*cis*-isoeugenol)	5912-86-7	04.004		0.30	0.13–0.36
2-Ethylphenol	90-00-6	04.070		0.29	0.27–0.31
2-Methylbenzofuran	4265-25-2	–		0.28	0.26–0.29
Indene	95-13-6	–		0.24	0.17–0.25
3-Methyl-1,2-cyclopentanedione (3-methylcyclopentan-1,2-dione)	765-70-8	07.056		0.17	0.11–0.26
3-Ethylphenol	620-17-7	04.021		0.16	0.15–0.20
2,3-Dimethyl phenol	526-75-0	04.065		0.16	0.15–0.16
Styrene	100-42-5	–		0.15	0.15–0.16
3,4-Dimethyl phenol	95-65-8	04.048		0.14	0.14–0.15
Hydroquinone	123-31-9	–		0.13	0.09–0.20
2-Methoxy-5-methylphenol	1195-09-1	–		0.12	0.12–0.13
Dihydrosyringenin	20736-25-8	–		0.12	0.09–0.16
Benzofuran	271-89-6	–		0.11	0.09–0.13
2,3-Butanedione (diacetyl)	431-03-8	07.052		0.08	0.05–0.10
Acetophenone	98-86-2	07.004		0.07	0.06–0.11
1,2,3-Trimethoxy-5-methyl benzene	6443-69-2	–		0.07	0.06–0.08
1-(4-Hydroxy-3-methoxyphenyl)-2-propanone	2503-46-0	–		0.06	0.04–0.07
Total				30.99	26.49–34.79[a]

Abbreviations: CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.

[a]The values given for Total are the lowest and the highest values of the sum of the components in the batches analysed.

The FEEDAP Panel notes that there are discrepancies between the analytical data on the volatile components submitted for the renewal of the additive and the data provided by the applicant at the time of the previous assessment (EFSA FEEDAP Panel, 2012). The applicant argued that the differences in the number of compounds identified (52 in the former vs. 41 in the current assessment) and in the relative concentrations are not due to a change in the manufacturing process, but rather in some changes in the gas chromatography–mass spectrometry/flame ionisation detector (GC–MS/FID) method used to identify and quantify the components. In the current dataset, volatiles were considered identified only when the chromatographic and mass spectrometric data matched those of the reference standards. The remaining compounds were considered tentatively identified or unidentified. The different concentrations analysed were ascribed to several changes in the gas chromatographic conditions (column temperature range, resulting in enhanced retention of compounds with high boiling point and polarity and better resolution) and in the use of FID to quantify the volatile components. The FEEDAP Panel notes that it is likely that the new characterisation data are more reliable than those submitted for the previous assessment. However, as the current method has not been evaluated by the EURL, the FEEDAP Panel is not in the position to conclusively comment on the composition of the additive.

In the new dataset, the presence of benzofuran (0.11%, range: 0.09%–0.13%) and styrene (0.15%, range 0.15%–0.16%) has been reported.

3.1.1.2 | *Characterisation of the non-volatile fraction*

The non-volatile fraction [calculated as 100 – water (wt %) – total volatiles (wt %)] accounts on average for 59.7 wt % (55–66.4 wt %) of the additive under assessment.

When the additive was dried at 350°C, the non-volatile fraction accounted for 24 wt % (20.0%–27.3%). The additive as such or after evaporation of the volatile fraction at 80°C was analysed by size exclusion chromatography (SEC). The molecular size of the additive was estimated at about 1.4 Kd. To further characterise the non-volatile fraction, after evaporation of the volatile fraction at 80°C the additive was subject to alkaline oxidation (with 20% H_2O_2 and 4% NaOH at 80°C for 15 min) to depolymerise the lignin polymer. The resulting mixture was analysed by GC–MS. A number of compounds (58) were tentatively identified, based on the comparison of their mass spectra with those from a MS-library, but not quantified.[14]

The FEEDAP Panel notes that the compounds identified are volatile degradation products obtained under oxidative conditions and do not necessarily represent the monomers in the non-volatile fraction. Therefore, the methodology applied was not considered adequate to characterise the non-volatile fraction, which remains uncharacterised.

3.1.1.3 | *Impurities*

In the authorising regulation, maximum limits are set for benzo[*a*]pyrene (< 10 μg/kg), benzo[*a*]anthracene (< 20 μg/kg) and for residual diethyl ether (< 2 mg/kg). In addition, the applicant set specifications for the content of cadmium (< 1 mg/kg), lead (< 5 mg/kg), mercury (< 1 mg/kg) and arsenic (< 3 mg/kg).

The applicant provided analytical data on the concentrations of cadmium, lead, mercury and arsenic and polycyclic aromatic compounds (PAHs) in six batches of the additive.[15] The contents of benzo[*a*]pyrene (2.2–4.7 μg/kg) and benzo[*a*]anthracene (6.9–15.2 μg/kg) were below the limits specified in the authorising regulation. The following PAHs were quantified: chrysene (4.9–11.2 μg/kg), benzo[*b*]fluoranthene (1.8–3.5 μg/kg), benzo[*k*]fluoranthene (0.7–1.4 μg/kg), benzo[*j*]fluoranthene (1.4–2.9 μg/kg), benzo[*g,h,i*]perylene (< 0.5–1.1 μg/kg), cyclopenta[*c, d*]pyrene (7.3–25.4 μg/kg), benzo[c]fluorene (44.7–75.7 μg/kg). The remaining PAHs dibenzo[*a, h*]pyrene, dibenzo[*a, l*]pyrene, dibenzo[*a, i*]pyrene, dibenzo[*a, e*]pyrene and 5-methylchrysene were below the LOQ of 1 μg/kg. Analytical data on the presence of residual diethyl ether were not provided. Cadmium (< 0.01 mg/kg), lead (< 0.05 mg/kg), mercury (< 0.005 mg/kg) and arsenic (< 0.1 mg/kg) were below the corresponding limit of quantification (LOQ) in all batches. Analytical data on the presence of dioxins and polychlorinated biphenyls were not provided.

The FEEDAP Panel considers that the level of cadmium, lead, mercury and arsenic do not raise safety concerns. The potential risk for the target species resulting from the presence of PAHs in the additive at the limits set in the regulation are assessed in Section 3.2.2.

3.1.2 | Physical properties of the additive

The additive is a viscous liquid of brown colour with a characteristic odour of concentrated smoke.

The additive is specified to have a density (at 20°C) of 1140–1160 kg/m^3 and a refractive index (20°C) of 1.50–1.70. The analysis of six batches of the additive[16] showed compliance with the new proposed specifications: density (at 20°C) 1144–1150 kg/m^3, refractive index (at 20°C) 1.568–1.579.

The additive is soluble in organic solvents, such as ethanol, diethyl ether, but not in water.

[14]Annex_08_non_volatile_AR.

[15]Annex_04_PAH_heavy metals_As_AR.

[16]Annex_01_Chemical characterisation_AR_AM.

3.1.3 | Stability

The shelf life of the additive (one batch) was studied when stored at 7–25°C and 60% relative humidity for 6 months and at 40°C for 3 and 6 months. The concentrations of the 41 volatile compounds were monitored. The results showed little evidence of degradation, with the largest relative deviations seen for the compounds of the lowest concentration.[17]

3.1.4 | Conditions of use

The additive is currently authorised for use in feed for dogs and cats at a maximum content of 40 mg/kg complete feed.

Under other provisions it is stated:

1. In the directions for use of the additive and premixture, indicate the storage and stability conditions.
2. For user safety: breathing protection and safety glasses should be worn during handling.
3. Labelling of premixtures, feed materials and compound feed containing the additive: the name of the additive shall be accompanied by the identification number.
4. The preparation may only contain technological additives and/or other substances or products intended to modify the physico-chemical characteristics of the active substance of the preparation and which are used in conformity with their own conditions of authorisation. Physicochemical and biological compatibility between the components of the preparation shall be ensured in relation to the effects desired.
5. The following information shall be set out on the label or accompanying documents of the additive: – the name and identification number of any technological additive contained in the preparation; – the level of any technological additive contained in the preparation where maximum contents are set in the corresponding authorisation; – the name of any substance or product contained in the preparation, indicated in descending order by weight.
6. The following information shall be set out on the label or accompanying documents of the premixture containing the additive: the name, identification number and level of any technological additive for which maximum contents are set in the corresponding authorisation.

The applicant did not request any change in the current conditions of use of the additive.

3.2 | Safety

The safety of the additive, previously referred to as Scansmoke SEF7525, for the target species and the users was evaluated in a former opinion (EFSA FEEDAP Panel, 2012). The FEEDAP Panel concluded that a concentration of 40 mg/kg complete feed would be safe for dogs and cats. No concern for genotoxicity was raised. In the absence of specific data relevant to the assessment of user safety, the additive was considered to be a possible skin, eyes and respiratory irritant based on the Safety Data Sheet provided by the applicant.

The additive is intended to be used only in feed for dogs and cats, and therefore, there is no need to perform an assessment of the safety for the consumers and the environment.

3.2.1 | Genotoxicity

In 2012 the FEEDAP Panel concluded that there was no concern regarding the genotoxicity of the additive (EFSA FEEDAP Panel, 2012), in line with the conclusions of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), which evaluated the same product (the smoke flavour Primary Product – Scansmoke SEF7525) based on the same dataset (EFSA CEF Panel, 2009). In that assessment, the CEF Panel considered that the negative outcomes of two in vivo genotoxicity studies (i.e. a micronucleus test without demonstration of bone marrow exposure and an unscheduled DNA synthesis (UDS) assay) were sufficient to rule out the concerns over in vitro genotoxicity identified in the mouse lymphoma assay.

Currently, however, the in vivo UDS assay is no longer recommended by EFSA, and the reliability and significance of negative results have to be carefully evaluated including evidence for target tissue exposure (EFSA Scientific Committee, 2017). In addition, for mixtures containing a substantial fraction of unidentified components, the EFSA Scientific Committee recommends that first the chemically defined substances are assessed individually for their potential genotoxicity using all available information, including read-across and quantitative structure–activity relationship (QSAR) considerations about their genotoxic potential (EFSA Scientific Committee, 2019a). Therefore, the potential genotoxicity of identified constituents is first considered. Then, in vitro and in vivo genotoxicity studies performed with the additive under assessment (whole mixture) are considered.

[17]Annex_09_Stability_AR.

In line with the requirements of the EFSA documents on genotoxicity (EFSA Scientific Committee, 2011, 2017, 2019a, 2021) and the EFSA guidance for the preparation of applications on smoke flavouring Primary Products (EFSA FAF Panel, 2021), new genotoxicity data were generated to support the renewal of the authorisation of Scansmoke SEF7525 for use in food. The new evidence, which included information on the genotoxicity of the individual components and data on the genotoxicity of the whole additive, has been evaluated by the EFSA Panel on Food Additives and Flavourings (FAF) in the opinion on the renewal of the authorisation of Scansmoke SEF7525 (SF-004) as smoke flavourings Primary Product (EFSA FAF Panel, 2023).

The FEEDAP Panel notes that the same genotoxicity dataset has been made available for the current assessment.

The outcome of the genotoxicity assessment made by the FAF Panel is summarised below.

3.2.1.1 | *Analysis of the genotoxic activity of the individual identified components*

The 41 identified components of the additive were evaluated individually for genotoxicity considering first the data available from the literature and then, in case of absence of relevant information from the literature, using in silico information. Twenty-nine out of the 41 identified and quantified components (see Table 1) have been already evaluated by EFSA and/or by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)/Council of Europe (CoE) as flavouring substances and the FAF Panel relied on these assessments to conclude on the absence of a genotoxic potential. For 10 components,[18] the FAF Panel concluded that there was no concern for genotoxicity based on literature data and in silico analyses performed applying (Q)SAR models and read-across (for details on the methodology, see EFSA FAF Panel, 2023). Concerning the remaining two components styrene (CAS No. 100-42-5; former FLAVIS No. [01.015]) and benzofuran (CAS No. 271-89-6), the FAF Panel noted that the available dataset indicated a potential concern for genotoxicity. However, appropriate follow-up studies were not available, and the information was not sufficient to rule out a potential safety concern for genotoxicity.

For these two substances, the FAF Panel applied the threshold of toxicological concern (TTC) concept (EFSA Scientific Committee, 2019b), in line with the evaluation scheme/principles of the EFSA guidance for the preparation of applications on smoke flavouring Primary Products (EFSA FAF Panel, 2021). When the exposure to genotoxic substances is very low, i.e. below the TTC value of 0.0025 μg/kg body weight (bw) per day for DNA-reactive mutagens and/or carcinogens, it can be concluded that there is a low probability of adverse effects. The estimated exposure to styrene and benzofuran via the Primary Product Scansmoke SEF7525 (for details, see EFSA FAF Panel, 2023) were at least a factor of 4440 above the TTC value of 0.0025 μg/kg bw per day. Therefore, conclusions could not be drawn based on the application of the TTC and additional data would be needed.

'Overall, the (FAF) Panel considered that further information on the genotoxicity of styrene and benzofuran would be needed to rule out the potential safety concern for genotoxicity of these substances. While for styrene conclusions on its genotoxic potential are expected from the CEP Panel (Panel on Food Contact Materials, Enzymes and Processing Aids), for benzofuran, appropriate genotoxicity studies conducted according to EFSA guidance (EFSA Scientific Committee, 2011, 2017, 2021) would be needed'.

3.2.1.2 | *Genotoxicity assessment of the additive (whole mixture)*

Considering that the additive under assessment contains a large fraction of unidentified volatile (7.4 wt %) and non-volatile (59.7 wt %) components, uncertainty also remains on the genotoxic potential of individual unidentified substances. In line with the guidance on the genotoxicity assessment of chemical mixtures, 'if a mixture contains a fraction of chemical substances that have not been chemically identified, experimental testing of the unidentified fraction should be considered as the first option or, if this is not feasible, testing of the whole mixture should be undertaken (EFSA Scientific Committee, 2019a)'.

The genotoxicity dataset with the whole mixture submitted for the current assessment and evaluated by the FAF Panel (EFSA FAF Panel, 2023) included: the genotoxicity studies[19] already evaluated in the previous opinion (EFSA FEEDAP Panel, 2012), and new in vitro and in vivo genotoxicity studies performed with the additive under assessment (whole mixture), i.e. an in vitro MN test,[20] an in vivo MN test[21] and an in vivo gene mutation test in transgenic rodents.[22] The additive induced gene mutation in vitro in mammalian cells, but not in bacterial cells, and negative results were reported in vivo in transgenic mice. Positive results were obtained in vitro for the induction of chromosomal damage in a micronucleus test, while no clastogenicity was observed in an in vitro chromosomal aberration test. In vivo, the Primary Product did not induce micronuclei in mice bone marrow erythrocytes. However, the FAF Panel considered this result of limited relevance because 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 (EFSA Scientific Committee, 2019a).

[18] 2,3-Dimethyl-2-cyclopenten-1-one, 2-methylbenzofuran, indene, styrene, hydroquinone, 2-methoxy-5-methylphenol, dihydrosyringenin, benzofuran, 1,2,3-trimethoxy-5-methyl benzene and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone.

[19] Annex_13_SEF7525_Ames_2005; Annex_14_SEF7525_MLA; Annex_15 SEF7525_CA_test_2005; Annex_16_SEF7525_Mouse_MNT_2005.

[20] Annex_17_MNvit_SEF7525_K100.

[21] Annex_18_MNinvivo_SEF7525_K173.

[22] Annex_19_DRF_TGR_SEF7525_K098 and Annex_20_TGR_SEF_7525_K099_Combined.

Therefore, based on the available dataset, no conclusion could be drawn on the genotoxicity of the whole mixture and additional data would be needed, as detailed in the FAF opinion (EFSA FAF Panel, 2023).

3.2.1.3 | *Overall conclusions on genotoxicity*

The FEEDAP Panel agrees with the conclusions of the FAF Panel that based on the available data, a potential concern for the genotoxicity of the additive under assessment remains and additional data would be needed to complete the assessment.

3.2.2 | Safety for the target species

3.2.2.1 | *Benzofuran and styrene*

By applying the same approach of the EFSA FAF Panel, the FEEDAP Panel applied the TTC approach, comparing the estimated exposure of the target animals to benzofuran and styrene with the threshold for DNA-reactive mutagens and/or carcinogens (EFSA Scientific Committee, 2019b).

At the maximum authorised use level in feed of 40 mg/kg, the content of benzofuran and styrene in feed would be up to 0.052 mg benzofuran/kg and 0.064 mg styrene/kg.

The highest benzofuran and styrene intake by dogs and cats were calculated at the proposed use levels of 40 mg/kg complete feed (EFSA FEEDAP Panel, 2017). 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 (EFSA Scientific Committee, 2019b). The results are shown in Table 2.

TABLE 2 Benzofuran and styrene intake (expressed as μg/kg bw per day) calculated at the proposed use level of 40 mg/kg complete feed and comparison with the threshold of toxicological concern (TTC) value of 0.0025 μg/kg bw per day for DNA-reactive mutagens and/or carcinogens.

	Benzofuran intake μg/kg bw per day	Ratio between exposure and TTC	Styrene intake μg/kg bw per day	Ratio between exposure and TTC
Dogs	0.985	394	1.212	485
Cats	1.182	473	1.455	582

All exposure estimates were at least a factor of 394 above the TTC value for DNA-reactive mutagens and/or carcinogens. Therefore, additional data would be needed to conclude on the genotoxicity of these substances and on the safety for the target species.

3.2.2.2 | *Additive under assessment (whole mixture)*

Since the additive under assessment contains a large fraction of unidentified volatile (7.4 wt %) and non-volatile (59.7 wt %) components, uncertainty remains on the genotoxic potential of individual unidentified substances. The whole mixture raises a potential concern for genotoxicity in vitro (see Section 3.2.1.2). Therefore, additional data would be needed to conclude on the genotoxicity of the whole mixture and on the safety for the target species.

3.2.2.3 | *Polycyclic aromatic hydrocarbons*

The EFSA FAF Panel performed a risk assessment of four representative PAHs, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene and chrysene (PAH4). For the sum of these congeners, a reference point (a benchmark dose lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) of 340 μg/kg bw per day) based on a carcinogenicity study is available (Culp et al., 1998, as reported in EFSA CONTAM Panel, 2008). The FAF Panel considered two concentration scenarios, assuming that: (i) benzo[a]pyrene and benzo[a]anthracene are both present in the additive at the specification limits of 10 μg/kg and 20 μg/kg, respectively, resulting in a sum of 30 μg/kg in the additive, whereas the concentration of benzo[b]fluoranthene and chrysene is set to zero; (ii) the four congeners are present at the highest reported value (benzo[a]anthracene, 15.2 μg/kg; benzo[a]pyrene, 4.7 μg/kg; benzo[b]fluoranthene, 3.5 μg/kg; and chrysene, 11.2 μg/kg), resulting in 34.6 μg/kg in the additive. When the estimated exposures to the four PAHs via the Primary Product (for details, see EFSA FAF Panel, 2023) were compared to the reference point, the margin of exposure (MOE) was far above the value of 10,000 for both scenarios.

The FEEDAP Panel performed a risk assessment of the PAHs present in the additive, considering the two scenarios identified by the EFSA FAF Panel.

At the maximum authorised use level in feed of 40 mg/kg, the content of benzo[a]pyrene and benzo[a]anthracene in feed calculated at the specification limits would be 0.0004 and 0.0008 μg/kg, respectively, and 0.0012 μg/kg for the sum. When considering the presence of the 4 PAHs at the highest reported value, the content in feed would be 0.0014 μg/kg.

The calculated PAHs intake by dogs and cats for the two scenarios were calculated at the proposed use levels of 40 mg/kg complete feed (EFSA FEEDAP Panel, 2017) and compared with the $BMDL_{10}$ value of 340 μg/kg bw per day. The results are shown in Table 3.

TABLE 3 PAHs intake (expressed as μg/kg bw per day) calculated at the proposed use level of 40 mg/kg complete feed and margin of exposure (MOE) calculated comparing the animal intake $BMDL_{10}$ of 340 μg/kg bw per day.

	Scenario 1: BaP + BaA at the highest specification		Scenario 2: Highest analysed value PAH4	
	PAHs intake		PAHs intake	
	μg/kg bw per day	MOE	μg/kg bw per day	MOE
Dogs	2.27×10^{-6}	1.50×10^{8}	2.62×10^{-6}	1.30×10^{8}
Cats	2.73×10^{-6}	1.25×10^{8}	3.15×10^{-6}	1.08×10^{8}

Abbreviations: BaA, benzo[a]anthracene; BaP, benzo[a]pyrene; Bw, body weight; MOE, margin of exposure; PAH4, mixture of benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene and chrysene.

The FEEDAP Panel concludes that the presence of PAHs in the additive is very unlikely to be of safety concern for the target species.

3.2.2.4 | *Conclusions on safety for the target species*

Considering that the additive under assessment contains benzofuran and styrene, which are substances of potential concern for genotoxicity, and that the whole mixture raises a potential concern for genotoxicity, the FEEDAP Panel is not in the position to conclude on the safety for cats and dogs.

3.2.3 | Safety for the user

No specific information was submitted. The applicant provided a safety data sheet[23] where potential hazards for skin and eye contact and respiratory exposure are mentioned.

The FEEDAP Panel concludes that the additive should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser.

Uncertainty remains on the genotoxic potential of benzofuran and styrene and of individual substances present in the unidentified volatile and non-volatile fractions of the mixture. When handling the additive, exposure of unprotected users to genotoxic substances may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

3.3 | Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the original conditions that would have an impact on the efficacy of the additive.

Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

4 | CONCLUSIONS

The applicant has provided data which showed that the additive currently on the market does not fully comply with the conditions of authorisation, but with newly proposed specifications for carbonyl compounds, total acids and phenols in the additive. The current dataset also showed differences in the relative concentrations of the compounds identified in the active substance compared to the values reported in the authorisation. According to the applicant, these differences are not due to a change in the manufacturing process but rather to different methods of analysis. As the current methods have not been evaluated by the EURL, the FEEDAP Panel is not in the position to conclusively comment on the new specifications and the composition of the additive.

Considering that the additive under assessment contains benzofuran and styrene, which are substances of potential concern for genotoxicity, and that the whole mixture raises a potential concern for genotoxicity, additional data would be needed to complete the assessment. Therefore, the FEEDAP Panel is not in the position to conclude on the safety for cats and dogs.

[23] Annex_27_SDS_PET_SEF7525.

The FEEDAP Panel concludes that the additive should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. When handling the additive, exposure of unprotected users to potential genotoxic substances may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

ABBREVIATIONS

BaA	benzo[a]anthracene
BaP	benzo[a]pyrene
BMD	Benchmark dose
$BMDL_{10}$	BMD lower confidence limit for a benchmark response of 10%
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CoE	Council of Europe
CONTAM	EFSA Panel on Contaminants in the Food Chain
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FAF	EFSA Panel on Food additives and Flavourings
FAO	Food Agriculture Organization
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FLAVIS No	FLAVIS number
FLAVIS	The EU Flavour Information System
GC–MS	gas chromatography–mass spectrometry
GC–MS/FID	gas chromatography–mass spectrometry/flame ionisation detector
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOQ	limit of quantification
MOE	margin of exposure
PAH4	mixture of benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene and chrysene
PAHs	polycyclic aromatic hydrocarbons
(Q)SAR	(Quantitative) Structure–Activity Relationship
SEC	size exclusion chromatography
TTC	threshold of toxicological concern
WHO	World Health Organization
wt	weight

ACKNOWLEDGEMENTS

The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): Montserrat Anguita, Jaume Galobart and Maria Vittoria Vettori.

CONFLICT OF INTEREST

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

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2023-00900

PANEL MEMBERS

Roberto Edoardo Villa, Giovanna Azimonti, Eleftherios Bonos, Henrik Christensen, Mojca Durjava, Birgit Dusemund, Ronette Gehring, Boet Glandorf, Maryline Kouba, Marta López-Alonso, Francesca Marcon, Carlo Nebbia, Alena Pechová, Miguel Prieto-Maradona, Ilen Röhe and Katerina Theodoridou.

REFERENCES

Culp, S. J., Gaylor, D. W., Sheldon, W. G., Goldstein, L. S., & Beland, F. A. (1998). A comparison of the tumours induced by coal tar and benzo[a]pyrene in a 2-year bioassay. *Carcinogenesis*, *19*, 117–124. https://doi.org/10.1093/carcin/19.1.117

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2009). Scientific Opinion on the safety of smoke flavour primary product – Scansmoke SEF7525. *EFSA Journal*, *7*(5), 1903. https://doi.org/10.2903/j.efsa.2009.1093

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). (2008). Scientific Opinion on polycyclic aromatic hydrocarbons in food. *EFSA Journal*, *6*(8), 724. https://doi.org/10.2903/j.efsa.2008.724

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings). (2021). Scientific Guidance for the preparation of applications on smoke flavouring primary products. *EFSA Journal*, *19*(3), 6435. https://doi.org/10.2903/j.efsa.2021.6435

EFSA FAF Panel (EFSA Panel name on Food Additives and Flavourings), Younes, M., Aquilina, G., Castle, L., Degen, G., Engel, K.-H., Fowler, P. J., Frutos Fernandez, M. J., Fürst, P., Gundert-Remy, U., Gürtler, R., Husøy, T., Manco, M., Moldeus, P., Passamonti, S., Shah, R., Waalkens-Berendsen, I., Wright, M., Benigni, R., … Mennes, W. (2023). Scientific Opinion on the renewal of the authorisation of Scansmoke SEF7525 (SF-004) as a smoke flavouring primary product. *EFSA Journal*, *21*(11), 1–50. https://doi.org/10.2903/j.efsa.2023.8366

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2012). Scientific Opinion on the safety and efficacy of Scansmoke SEF7525 (smoke flavourings) for cats and dogs. *EFSA Journal*, *10*(6), 2729. https://doi.org/10.2903/j.efsa.2012.2729

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2017). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, *15*(10), 5021. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Kouba, M., Fašmon Durjava, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Anguita, M., Galobart, J., … Innocenti, M. L. (2021a). Guidance on the renewal of the authorisation of feed additives. *EFSA Journal*, *19*(1), 6340. https://doi.org/10.2903/j.efsa.2021.6340

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2021b). *General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic*. https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf

EFSA Scientific Committee. (2011). Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA Journal*, *9*(9), 2379. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee, Hardy, A., Benford, D., Halldorsson, T., Jeger, M., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Silano, V., Solecki, R., Turck, D., Younes, M., Aquilina, G., Crebelli, R., Gürtler, R., … Schlatter, J. (2017). Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. *EFSA Journal*, *15*(12), 5113. https://doi.org/10.2903/j.efsa.2017.5113

EFSA Scientific Committee, More, S., Bampidis, V., Benford, D., Boesten, J., Bragard, C., Halldorsson, T., Hernandez-Jerez, A., Hougaard-Bennekou, S., Koutsoumanis, K., Naegeli, H., Nielsen, S. S., Schrenk, D., Silano, V., Turck, D., Younes, M., Aquilina, G., Crebelli, R., Gürtler, R., … Schlatter, J. (2019a). Statement on the genotoxicity assessment of chemical mixtures. *EFSA Journal*, *17*(1), 5519. https://doi.org/10.2903/j.efsa.2019.5519

EFSA Scientific Committee, More, S. J., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T. I., Hernandez-Jerez, A. F., Hougaard, B. S., Koutsoumanis, K. P., Machera, K., Naegeli, H., Nielsen, S. S., Schlatter, J. R., Schrenk, D., Silano, V., Turck, D., Younes, M., Gundert-Remy, U., Kass, G. E. N., … Wallace, H. M. (2019b). Guidance on the use of the threshold of toxicological concern approach in food safety assessment. Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. *EFSA Journal*, *17*(6), 5708. https://doi.org/10.2903/j.efsa.2019.5708

EFSA Scientific Committee, More, S. J., Bampidis, V., Bragard, C., Halldorsson, T. I., Hernandez-Jerez, A. F., Hougaard, B. S., Koutsoumanis, K. P., Machera, K., Naegeli, H., Nielsen, S. S., Schlatter, J. R., Schrenk, D., Silano, V., Turck, D., Younes, M., Aquilina, G., Bignami, M., Bolognesi, C., … Benford, D. (2021). Scientific Opinion on the guidance on aneugenicity assessment. *EFSA Journal*, *19*(8), 6770. https://doi.org/10.2903/j.efsa.2021.6770

How to cite this article: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Villa, R. E., Azimonti, G., Bonos, E., Christensen, H., Durjava, M., Dusemund, B., Gehring, R., Glandorf, B., Kouba, M., López-Alonso, M., Marcon, F., Nebbia, C., Pechová, A., Prieto-Maradona, M., Röhe, I., Theodoridou, K., Bastos, M. L., Brantom, P.,… Manini, P. (2024). Assessment of the feed additive consisting of a preparation containing a smoke flavouring extract for cats and dogs for the renewal of the authorisation (Azelis Denmark A/S). *EFSA Journal*, *22*(10), e9030. https://doi.org/10.2903/j.efsa.2024.9030