Safety and efficacy of feed additives prepared from *Piper nigrum* L.: black pepper oil and black pepper oleoresin for use in all animal species and a supercritical extract for use in dogs and cats (FEFANA asbl)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pečová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Johannes Westendorf, Paola Manini, Fabiola Pizzo and Birgit Dusemund

Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of feed additives prepared from *Piper nigrum* L.: black pepper oil and black pepper oleoresin for all animal species and a supercritical extract of black pepper for use in dogs and cats. The Panel concludes that black pepper oil is safe in complete feed up to 5 mg/kg for chickens for fattening and other growing poultry, 8 mg/kg for laying hens and other laying/breeding birds kept for egg production/reproduction, 7 mg/kg for turkeys for fattening, 9.5 mg/kg for piglets and other growing *Suidae*, 11.5 mg/kg for pigs for fattening, 14 mg/kg for sows and dairy cows (and other dairy ruminants), 8.5 mg/kg in rabbits and 20 mg/kg in veal calves, cattle for fattening (and other growing ruminants), sheep, goats, horses, salmonids (and other fin fish), dogs, cats and ornamental fish. For all the other species, the additive is considered safe at 5 mg/kg complete feed. The supercritical extract of black pepper is safe up to the maximum proposed use levels in complete feed of 1.5 mg/kg for cats and dogs. The black pepper oleoresin is safe in complete feed up to 12.5 mg/kg for veal calves, 11.5 for cattle for fattening and other growing ruminants, sheep/goats and horses, 14 mg/kg for dogs 13.5 for mg/kg for salmonids and other fin fish and 51.5 for ornamental fish. For the other species, the calculated safe concentrations in complete feed are 1 mg/kg for chickens for fattening and other growing poultry, 1.4 mg/kg for laying hens and other laying/breeding birds kept for egg production/reproduction, 1.3 for turkeys for fattening, 1.7 mg/kg for piglets and other growing *Suidae*, 2 mg/kg for pigs for fattening, 2.5 mg/kg for sows, 2.4 mg/kg for dairy cows and other dairy ruminants, 1.5 mg/kg for rabbits, 3.8 mg/kg for cats. For all the other species, the additive is considered safe at 1 mg/kg complete feed. No concerns for consumers and environment were identified following the use of the additives at the use levels considered safe for the target animals. The additives are irritant to skin and eyes, and act as dermal and respiratory sensitisers. The additives are recognised to flavour food and since their function in feed would be essentially the same, no further demonstration of efficacy is necessary.

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Keywords: Extract, oleoresin, sensory additives, flavouring compounds, *Piper nigrum* L., safety, efficacy

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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 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. In addition, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)\(^2\) for authorisation/re-evaluation of 18 preparations (cassia oil, cassia bark extract (sb), camphor oil, cinnamon oil, cinnamon bark oleoresin, cinnamon tincture, laurel leaves oil, laurel leaves extract/oleoresin, litsea berry oil, boldo extract (wb), boldo tincture, ylang-ylang oil, mace oil, nutmeg oil, nutmeg oleoresin, kawakawa tincture, pepper oil and pepper oleoresin) belonging to botanically defined group (BDG) 6 – Laurales, Magnoliales, Piperales, when used as a feed additive for all animal species (category: sensory additives; functional group: flavouring compounds). During the assessment, the applicant withdrew the applications for eight preparations.\(^3\) These preparations were deleted from the register of feed additives.\(^4\) In addition, during the course of the assessment, the application was split and the present opinion covers only two out of the initial 18 preparations under application: pepper oil and pepper oleoresin from *Piper nigrum* L. for all animal species. During the assessment, the applicant clarified that black pepper oil (steam distilled) and the supercritical extract of black pepper fall into the definition “pepper oil”. The three preparations from *P. nigrum* L. will be assessed separately. During the assessment, for the supercritical extract of black pepper, the applicant requested a change in the species limiting the application for authorisation to dogs and cats.

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 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 3 January 2011.

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 products black pepper oil, supercritical extract of black pepper and black pepper oleoresin from the whole or broken unripe fruits of *Piper nigrum* L., when used under the proposed conditions of use (see Sections 3.2.1.3, 3.3.1.3 and 3.4.1.3).

The remaining eight preparations belonging to botanically defined group (BDG) 6 - Laurales, Magnoliales, Piperales under application are assessed in separate opinions.

1.2. **Additional information**

The two preparations under assessment, namely black pepper oil and black pepper oleoresin from *Piper nigrum* L., are currently authorised as feed additives according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined). They have not been assessed as feed additives in the EU. Two different additives fall into the definition ‘black pepper oil’, obtained by steam distillation or by carbon dioxide (CO\(_2\)) supercritical extraction.

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1 Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, pp. 29.

2 On 13/03/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1,050 Brussels, Belgium.

3 On 8 October 2020, EFSA was informed about the withdrawal of the applications on cassia bark extract (sb), cinnamon bark oleoresin, laurel leaves extract/oleoresin, mace oil, nutmeg oleoresin, boldo extract (wb), boldo tincture and kawakawa tincture.

4 Register of feed additives, Annex II, withdrawn by OJ L162, 10.05.2021, pp. 5.

5 Technical dossier/Supplementary information (May 2021)/SIn_reply_pepper_oil_black.
‘Pepper (Piperis fructus)’ is described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020). It is defined as the dried ripe or nearly ripe fruit of *Piper nigrum* L. with an unbroken pericarp (black pepper) or with the outer layers of the pericarp removed (white pepper).

Many of the individual components of black pepper oil, supercritical extract of black pepper and black pepper oleoresin have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) and the EFSA Panel on Food Additives and Flavourings (FAF). The list of flavouring compounds currently authorised for food⁶ and feed⁷ uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000⁸ and the corresponding EFSA opinion is given in Table 1.

**Table 1:** Flavouring compounds already assessed by EFSA as chemically defined flavourings, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/2000, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding EFSA opinion

| CG  | Chemical Group                                                                 | Product – EU register name (common name) | FLAVIS No  | EFSA or JECFA opinion,(* ) | Year      |
|-----|-------------------------------------------------------------------------------|-----------------------------------------|------------|----------------------------|-----------|
| 01  | Straight-chain primary aliphatic alcohols/aldehydes/esters containing saturated alcohols and esters containing saturated aldehydes | Hexadecanoic acid (palmitic acid)       | 08.014     | 2013                       |           |
|     |                                                                               | Propyl isovalerate                      | 09.448     | JECFA, 1998                |           |
| 03  | a, ß-Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/esters, esters and esters | Phytol                                 | 02.204     | 2010a, CEF                 |           |
| 06  | Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters containing tertiary alcohols ethers | Linalool                               | 02.013     | 2012a                      |           |
|     |                                                                               | ß-Terpineol                             | 02.014     |                            |           |
|     |                                                                               | Nerolidol                              | 02.018     |                            |           |
|     |                                                                               | 2-(4-Methylphenyl)propan-2-ol           | 02.042     |                            |           |
|     |                                                                               | 4-Terpinenol                           | 02.072     |                            |           |
|     |                                                                               | ß-Elemol                               | 02.149     | 2015a, CEF                 |           |
| 10  | Secondary aliphatic saturated or unsaturated alcohols, ketones, ketals and esters with a second secondary or tertiary oxygenated functional group | Butane-2,3-diol                        | 02.133     | 2014a, CEF                 |           |
|     |                                                                               | 4-Hydroxy-4-methylpentan-2-one         | 07.165     |                            |           |
| 16  | Aliphatic and alicyclic ethers                                                | 1,8-Cineole                            | 03.001     | 2012b, 2021                |           |
| 23  | Benzyl alcohols/aldehydes/esters/acetals                                       | Piperonal                               | 05.016     | 2012c                      |           |
| 28  | Pyridine and pyrrole derivatives                                               | Piperine                               | 14.003     | 2016a                      |           |
| 30  | Miscellaneous substances                                                       | Piperidine                             | 14.010     | 2015b, CEF                 |           |
|     |                                                                               | Deca-(2E,4E)-dienoic acid isobutyl-amide | 16.091     |                            |           |
| 31  | Aliphatic and aromatic hydrocarbons and acetalts containing saturated aldehydes | Limonone¹(²)                           | 01.001     | 2008, AFC                  |           |
|     |                                                                               | 1-Isopropyl-4-methylbenzene (p-cymene)  | 01.002     |                            | 2015      |

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⁶ Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, pp. 1.

⁷ European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: [https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf](https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf)

⁸ 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.
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 in support of the authorisation request for the use of three preparations from *P. nigrum* L. as feed additives.

The FEEDAP Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 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.

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to refer to the data submitted for the assessment of chemically defined flavourings (dossiers, publications and

(*): FEEDAP opinion unless otherwise indicated.

(1): Evaluated for use in food. According to Regulation (EC) 1,565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.

(2): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008). d-Limonene [01.045] and l-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015).

(3): *b-Ocimene [01.018]*, as a mixture of (E)- and (Z)-isomers, containing 50–70% (E)-isomer and 17–17% (Z)-isomer, was evaluated.

(4): Evaluated applying the ‘Procedure’ described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010b).

| CG | Chemical Group | Product – EU register name (common name) | FLAVIS No | EFSA or JECFA opinion, (*) | Year |
|----|----------------|------------------------------------------|-----------|----------------------------|------|
| 32 | Epoxides       | β-Caryophyllene epoxide                  | 01.041    | 2014b, CEF                 |      |
| 16 | Epoxides       | 3,7-Dimethyl-1,3,6-octatriene             | 01.014    |                            |      |
| 16 | Epoxides       | 3,7,10-Humulatriene                      | 01.043    |                            |      |
| 16 | Epoxides       | 4(10)-Thujene (sabinene)                | 01.059    | 2015c, CEF                 |      |
| 16 | Epoxides       | cis-3,7-Dimethyl-1,3,6-octatriene (cis-β-Ocimene) | 01.064 |                            |      |
| 16 | Epoxides       | β-Farnesene                             | 01.041    |                            |      |
| 16 | Epoxides       | β-Germacrene                            | 01.042    |                            |      |
| 16 | Epoxides       | β-Elemene                               | 01.039    |                            |      |
| 16 | Epoxides       | β-Cubebene                              | 01.030    |                            |      |
| 16 | Epoxides       | β-Bisabolene                            | 01.028    |                            |      |
| 16 | Epoxides       | α-Cadinene                              | 01.021    | 2011, CEF                  |      |
| 16 | Epoxides       | 1(5),11-Guaiadiene                      | 01.023    |                            |      |
| 16 | Epoxides       | 3,7-Dimethyl-1,3,6-octatriene (β-ocimene) | 01.018 |                            |      |
| 16 | Epoxides       | δ-3-Carene                              | 01.029    |                            |      |
| 16 | Epoxides       | 3,7,10-Humulatriene (β-ocimene)         | 01.047    |                            |      |
| 16 | Epoxides       | 4(10)-Thujene (sabinene)               | 01.059    | 2015c, CEF                 |      |
| 16 | Epoxides       | cis-3,7-Dimethyl-1,3,6-octatriene (cis-β-Ocimene) | 01.064 |                            |      |
| 16 | Epoxides       | β-Farnesene                             | 01.041    |                            |      |
| 16 | Epoxides       | β-Germacrene                            | 01.042    |                            |      |
| 16 | Epoxides       | β-Elemene                               | 01.039    |                            |      |
| 16 | Epoxides       | β-Cubebene                              | 01.030    |                            |      |
| 16 | Epoxides       | β-Bisabolene                            | 01.028    |                            |      |
| 16 | Epoxides       | α-Cadinene                              | 01.021    | 2011, CEF                  |      |
| 16 | Epoxides       | 1(5),11-Guaiadiene                      | 01.023    |                            |      |
| 16 | Epoxides       | 3,7-Dimethyl-1,3,6-octatriene (β-ocimene) | 01.018 |                            |      |
| 16 | Epoxides       | δ-3-Carene                              | 01.029    |                            |      |

(1): Evaluated for use in food. According to Regulation (EC) 1,565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.

(2): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008). d-Limonene [01.045] and l-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015).

(3): *β-Ocimene [01.018]*, as a mixture of (E)- and (Z)-isomers, containing 50–70% (E)-isomer and 17–17% (Z)-isomer, was evaluated.

(4): Evaluated applying the ‘Procedure’ described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010b).

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 in support of the authorisation request for the use of three preparations from *P. nigrum* L. as feed additives.

The FEEDAP Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 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.

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to refer to the data submitted for the assessment of chemically defined flavourings (dossiers, publications and

9 FEED dossier reference: FAD-2010-0218.
unpublished reports) for the risk assessment of preparations belonging to BDG 6, including the current ones under assessment.10

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical markers in botanically defined flavourings from Group 06 – Laurales, Magnoliinales, Piperales. During the assessment, upon request from European Commission and EFSA, the EURL issued two amendments of the original report.11 For the additives under assessment, pepper oil black and pepper oleoresin black, the evaluation of the method of analysis is included in the second amendment. The Executive Summary of the EURL report can be found in Annex A.12

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of three preparations from P. nigrum L. is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, online version), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA SC, 2019b), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA SC, 2019c).

3. Assessment

The three additives under assessment obtained from fruits of Piper nigrum L. by different manufacturing methods are:

- black pepper oil.
- supercritical extract of black pepper.
- black pepper oleoresin.

The three preparations are intended for use as sensory additives (functional group: flavouring compounds) in feed and water for drinking for all animal species (black pepper oil and black pepper oleoresin) and for cats and dogs (supercritical extract of black pepper).

3.1. Origin and extraction of the three additives

Piper nigrum L. is a perennial climbing vine belonging to the family Piperaceae. It is native to the Malabar coast of India but is now cultivated commercially in many countries in South-East Asia, and in China, Ethiopia and Brazil. Its dried fruits, commonly referred to as peppercorns, are the source of the most widely and frequently used of all spices. Green peppercorns are prepared from the unripe fruit, black peppercorns are the untreated dried ripe fruit and white peppercorns, the ripe fruit with the thin dark outer layer removed. Black pepper has a long history of use in traditional medicine, particularly for digestive ailments.

10 Technical dossier/Supplementary information/Letter dated 29/04/2021.
11 Preparations included in the first amendment: ylang ylang oil, camphor white oil and cinnamon tincture; preparations included in the second amendment: nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black.
12 The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0218_en
13 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.
14 Available at: https://www.efsa.europa.eu/en/microstrategy/botanical-summary-report
Black pepper oil

This preparation is the essential oil obtained by steam distillation. Briefly, steam is passed through the plant material (dried and crushed unripe fruits). The steam carries up the volatile constituents which are then condensed. The essential oil is then separated from water by decantation.

Supercritical extract of black pepper

The second additive is obtained by CO₂ supercritical extraction from the dried and crushed unripe fruits (with no additional solvent addition).

Black pepper oleoresin

This preparation is produced by organic solvent extraction of the dried unripe fruits. Solvent extraction with ethyl acetate and/or hexane or hexane and acetone may be preceded by steam distillation to separately recover the volatile fraction (the essential oil). After a period of extraction, the solvent is removed by filtration or distillation and recycled. The residual dry matter is then standardised by addition of propylene glycol, and sometimes with glycerides of fatty acids.15

3.2. Black pepper oil

3.2.1. Characterisation

The essential oil under assessment is a colourless or light coloured (yellow, green, blue) clear mobile liquid with a spicy and woody odour. In five batches of the additive (originating from Sri Lanka), the specific gravity ranged between 0.872 and 0.879 (specification: 0.861–0.867) and the refractive index between 1.481 and 1.433 (specification: 1.475–1.490) and the specific optical rotation (nine batches) between –10.36 and –16.00 (specification: –17 and –8). Black pepper oil is identified with the single Chemical Abstracts Service (CAS) number 8006-82-4, the European Inventory of Existing Chemical Substances (EINECS) number 284-524-717 and the Flavor Extract Manufacturers Association (FEMA) number 2845.

The product specifications are based on the standards developed by the International Organisation for Standardization (ISO) 3061:2008 for black pepper oil (Piper nigrum L.),18 which were adapted to reflect the concentrations of the main volatile components, analysed by gas-chromatography with flame ionisation detection (GC-FID) and expressed as % of gas chromatographic peak area (% GC area). These components are β-caryophyllene, limonene, 4(10)-thujene (herein referred to as sabinene) and pin-2(3)-ene (herein referred to as α-pinene). Analysis of five batches for each product analysed by GC-FID showed compliance with these specifications. When analysed by gas chromatography–mass spectrometry (GC–MS), these four compounds account for 56.3 on average (ranging 54.1–58.7%), of the % GC area (Table 2).

Table 2: Major constituents of black pepper oil from the fruits of Piper nigrum L. based on analysis of five batches. The content of each constituent is expressed as the area percentage of the corresponding chromatographic peak (% GC area), assuming the sum of the chromatographic areas of all detected peaks as 100%

| Constituent                  | EU register name | CAS No  | FLAVIS No | % GC area | Specification | Mean(a) | Range   |
|------------------------------|------------------|---------|-----------|-----------|---------------|---------|---------|
| β-Caryophyllene              |                  | 87-44-5 | 01.007    | 12.0–40.0 | 25.6          | 25.1–26.0 |
| Limonene                     |                  | 138-86-3| 01.001    | 7.0–20.0  | 12.5          | 11.5–13.4|
| Sabinene (4(10)-thujene)     |                  | 3387-41-5| 01.059    | 4.0–17.0  | 9.4           | 9.1–9.6  |
| α-Pinene (pin-2(3)-ene)      |                  | 80-56-8 | 01.004    | 2.5–16.0  | 8.9           | 8.4–9.7  |
| Total                        |                  |         |           |           | 56.3          | 54.1–58.7|

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers. (a): Mean calculated on five batches.

15 Technical dossier/Supplementary information (January 2022)/Annex VII, Annex_VIII, Annex_IX.
16 Technical dossier/Supplementary information (May 2021)/Annex I.
17 In the ECHA website, this EINECS number is associated with the CAS number 84929-41-9 which corresponds to a wider category of Piper nigrum preparations.
18 Technical dossier/Supplementary information (May 2021)/Annex VII Pepper oil black ISO.
The applicant provided the full characterisation of the five batches by GC-MS and GC-FID. In total, 44 peaks were identified and accounted on average for 99.94% (99.88–99.96%) of the % GC area. Besides the four compounds indicated in the product specifications, 38 were detected at individual levels > 0.1% and are listed in Table 3. These 42 compounds (including those in the specifications) for black pepper oil together account on average for 99.73% (94.105.92%) of the % GC area. The remaining two compounds, accounting for 0.165%, are 1,8-cineole [03.001] and α-gurjunene. Based on the available data on the characterisation, black pepper oil is considered a fully defined mixture.

Table 3: Other constituents of the black pepper oil from the fruits of *Piper nigrum* L. accounting for > 0.1% of the composition (based on the analysis of five batches for each product) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

| Constituent | CAS No | FLAVIS No | % GC area black pepper oil Mean(a) | Range |
|-------------|--------|-----------|-----------------------------------|-------|
| β-Pinene (pin-2(10)-ene) | 127-91-3 | 01.003 | 7.76 | 7.57-7.88 |
| δ-3-Carene | 13466-78-9 | 01.029 | 7.27 | 6.87-7.55 |
| α-Copaene | 3856-25-5 | – | 4.60 | 4.44-4.75 |
| δ-Elemene | 20307-84-0 | 01.039 | 3.08 | 3.04-3.17 |
| Myrcene | 123-35-3 | 01.008 | 2.00 | 1.76-2.17 |
| β-Phellandrene | 555-10-2 | 01.055 | 1.95 | 1.72-2.14 |
| α-Phellandrene | 99-83-2 | 01.006 | 1.91 | 1.48-2.18 |
| 3,7,10-Humulatriene | 6753-98-6 | 01.043 | 1.72 | 1.66-1.83 |
| α-Cadinene | 29350-73-0 | 01.021 | 1.38 | 1.29-1.56 |
| β-Bisabolene | 495-61-4 | 01.028 | 1.37 | 1.34-1.44 |
| α-Thujene | 286705-2 | – | 1.32 | 1.19-1.52 |
| α-Murolene | 1020808-7 | 01.052 | 0.77 | 0.70-0.92 |
| 1-Isopropyl-4-methylbenzene (p-cymene) | 99-87-6 | 01.002 | 0.58 | 0.46-0.75 |
| Terpinolene | 586-62-9 | 01.005 | 0.57 | 0.48-0.63 |
| β-Caryophyllene epoxide | 1139-30-6 | 16.043 | 0.53 | 0.26-0.93 |
| 4-Terpinenol | 562-74-3 | 02.072 | 0.53 | 0.37-0.78 |
| Linalool | 78-70-6 | 02.013 | 0.53 | 0.51-0.54 |
| Bicyclogermacrene | 67650-90-2 | – | 0.52 | 0.49-0.55 |
| Germacr-1(10),4(14),5-triene | 23986-74-5 | 01.042 | 0.45 | 0.40-0.48 |
| β-Cubebe ne | 13744-15-5 | 01.030 | 0.45 | 0.39-0.49 |
| β-Elemene | 33880-83-0 | – | 0.42 | 0.35-0.53 |
| Camphene | 79-92-5 | 01.009 | 0.34 | 0.32-0.37 |
| γ-Terpinene | 99-85-4 | 01.020 | 0.33 | 0.28-0.41 |
| trans-Murol-5-en-4-α-ol | – | – | 0.29 | 0.24-0.35 |
| α-Cubebe ne | 17699-14-8 | – | 0.29 | 0.26-0.32 |
| β-Selinene | 17066-67-0 | – | 0.25 | 0.20-0.29 |
| trans-3,7-Dimethyl-1,3,6-octatriene | 3779-61-1 | – | 0.24 | 0.18-0.27 |
| Cyclosativene | 22469-52-9 | – | 0.20 | 0.19-0.21 |
| trans-Sabinene hydrate | 17699-16-0 | – | 0.19 | 0.18-0.19 |
| δ-Cadinol | 19435-97-3 | – | 0.18 | 0.14-0.24 |
| Isoterpinolene | 586-63-0 | – | 0.16 | 0.14-0.18 |
| Alismol | 87827-55-2 | – | 0.15 | 0.09-0.24 |
| cis-Sabinene hydrate | 15537-55-0 | – | 0.14 | 0.12-0.17 |

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19 Technical dossier/Supplementary information (May 2021)/Annex_III_SIn_Reply Pepper oil black chromatograms.
The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *Piper nigrum* L. and its preparations. Piperidine alkaloids (including piperine, piperidine, piperlonguminine) are reported as potential substances of toxicological concern in EFSA Compendium of Botanicals and BELFRIT list for the fruits of species *Piper nigrum* L. The presence of piperidine alkaloids (e.g. piperine, piperidine, piperlonguminine) in pepper fruits is also mentioned in the Commentary to the European Pharmacopoeia (EUPh Commentary, 2020). The applicant has performed a quantitative analysis on five batches of black pepper oil: the content of piperine measured by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) was on average < 0.0005%.

Based on one reference (Wrba et al., 1992), the occurrence of safrole is also reported in the EFSA Compendium, but not quantified. However, safrole was not identified in any batches analysed of black pepper oil (limit of detection, LOD 3 mg/kg).

### 3.2.1.1. Impurities

The applicant makes reference to the ‘periodic testing’ of some representative flavourings premixtures for mercury, cadmium, lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorous pesticides, aflatoxin B1, B2, G1, G2 and ochratoxin A. However, no data have been provided in the presence of these impurities. Since black pepper oil is produced by steam distillation, the likelihood of any measurable carry-over of the above-mentioned elements is low except for mercury.

### 3.2.1.2. Shelf-life

The typical shelf-life of the additive is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light). However, no data supporting this statement were provided.

### 3.2.1.3. Conditions of use

Black pepper oil is intended to be added to feed and water for drinking for all animal species without a withdrawal time. The maximum proposed use levels in complete feed for the selected target species are reported in Table 4. No use level has been proposed by the applicant for the use in water for drinking.

### Table 4: Conditions of use for the black pepper oil: maximum proposed use levels in complete feed for the different target species

| Use level (mg/kg feed) |
|------------------------|
| Chickens for fattening  | 5 |
| Laying hens             | 8 |
| Turkeys for fattening   | 7 |

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20 Technical dossier/Supplementary information (May 2021)/Literature search.
21 Available at: https://www.efsa.europa.eu/en/microstrategy/botanical-summary-report.
22 Technical dossier/Supplementary information (May 2021)/Annex IV.
23 Technical dossier/Section II.
3.2.2. Safety

The assessment of safety of black pepper oil is based on the maximum use levels proposed by the applicant.

Many of the components of the mixture, accounting for about 83.5% of the GC peak areas, have been previously assessed and considered safe at certain concentrations for use as flavourings, and are currently authorised for use in food without limitations and for use in feed at individual use levels higher than those resulting from the intended use of the essential oil in feed. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Six compounds, 1,8-cadinene [01.021], 1,8-cubebene [01.030], germacr-1(10),4(14),5-triene [01.042], 3,7,10-humulatriene [01.043], 1,6-muurolene [01.052] and 1,8-phellandrene [01.055], were evaluated in FGE25.Rev2 by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on foods (EFSA CEF Panel, 2010b). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional toxicity data (EFSA CEF Panel, 2011). In the absence of such toxicological data, the CEF Panel was unable to complete its assessment. As a result, these compounds are no longer authorised for use as flavourings in food. For these compounds, the FEEDAP Panel applies the approach recommended in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a).

Eighteen compounds, representing 11.4% of the mixture, were not previously evaluated as food or feed flavourings. The FEEDAP Panel notes that 15 of these compounds (10.9%) are aliphatic mono- or sesqui-terpenes structurally related to flavourings already assessed in CG 8 and CG 31 and a similar metabolic and toxicological profile is expected. Because of their lipophilic nature, they are expected to be rapidly absorbed from the gastro-intestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2015, 2016b,c).

The additive contains trans-muurol-5-en-4-α-ol, alismol and 1,8-cadinol which are structurally similar to feed flavouring additives allocated to CG 6 that have been evaluated by EFSA as safe for consumers (EFSA FEEDAP Panel, 2012a). The genotoxic potential of trans-muurol-5-en-4-α-ol, alismol and 1,8-cadinol was investigated (gene mutation, chromosomal damage) using the QSAR Toolbox (V.4.4.1). No alerts were found for alismol and 1,8-cadinol. For trans-muurol-5-en-4-α-ol, structural alert was due to the presence of the vinyl/alllyl alcohol group. For this compound, the mutagenicity (Ames test) prediction was made by read-across analyses of data available for similar substances (i.e. analogues obtained by categorisation). Categories were defined using general mechanistic and endpoint profilers as well as empirical profilers. Mutagenicity read-across-based predictions were found consistently negative for all categories of analogues. On this basis, the alert raised for trans-muurol-5-en-4-α-ol was discounted.

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| Compound                | Use level (mg/kg feed) |
|-------------------------|------------------------|
| Piglets                 | 9.5                    |
| Pigs for fattening      | 11.5                   |
| Sows                    | 14                     |
| Veal calves (milk replacer) | 20                  |
| Cattle for fattening    | 20                     |
| Dairy cows              | 14                     |
| Sheep/goat              | 20                     |
| Horse                   | 20                     |
| Rabbit                  | 8.5                    |
| Fish                    | 20                     |
| Dogs                    | 20                     |
| Cats                    | 20                     |
| Ornamental fish         | 20                     |

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24 α-copaene, α-thujene, bicyclogermacrene, β-elemene, α-cubebene, β-selinene, trans-3,7-dimethyl-1,3,6-octatriene, cyclosativene, trans-Sabinene hydrate, isoterpinolene, cis-Sabinene hydrate, α-selinene, β-copaene, trans-β-farnesene and α-gurjunene.

25 Technical dossier/Supplementary information (May 2021)/Annex X.
3.2.2.1. Safety for the target species

Tolerance studies and/or toxicological studies made with the essential oil under application were not submitted.

In the absence of toxicological data with the additive under assessment, the approach to the safety assessment of a mixture whose individual components are known is based on the safety assessment of each individual component (component-based approach). This approach requires that the mixture is sufficiently characterised. The individual components can be grouped into assessment groups, based on structural and metabolic similarity. The combined toxicity can be predicted using the dose addition assumption within an assessment group, taking into account the relative toxic potency of each component.

As the additive under assessment is a fully defined mixture (> 99% of the components were identified, see Section 3.2.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species.

Based on considerations related to structural and metabolic similarities, the components of black pepper oil were allocated to five assessment groups, corresponding to the chemical groups (CGs) 6, 8, 16, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000. For CG 31 (‘aliphatic and aromatic hydrocarbons’), the application of subassessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 is applied (EFSA CEF Panel, 2015c,d). The allocation of the components to the (sub) assessment groups is shown in Table 5 and in the corresponding footnote.

For each component in the assessment group, exposure in target animals was estimated considering the use levels in feed, the percentage of the component in the oil and the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b). Default values on body weight are used to express exposure in terms of mg/kg bw per day. The intake levels of the individual components calculated for chickens for fattening, the species with the highest ratio of feed intake/body weight gain per day, are shown in Table 5.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification. For some components in each assessment group, toxicological data were available to derive no observed adverse effect level (NOAEL) values. Structural and metabolic similarities among the components in the assessment groups were evaluated to explore the application of read-across allowing extrapolation from a known NOAEL of a component of an assessment group to the other components of the group with no available NOAEL or, if sufficient evidence were available for members of a (sub-)assessment group, to derive a (sub-)assessment group NOAEL.

Toxicological data for subchronic studies, from which NOAEL values could be derived, were available for linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a), 1,8-cineole in CG 16 (EFSA FEEDAP Panel, 2012b, 2021), myrcene [01.008], p-cymene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016b) and for β-caryophyllene oxide [16.043] in CG 32 (EFSA CEF Panel, 2014b).

Considering the structural and metabolic similarities, for the subgroup of terpinyl derivatives in CG 6, i.e. α-terpineol [02.014] and 4-terpinenol [02.072], the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol[27] [02.230] and limonene [01.001].

Similarly, the NOAELs for the representative compounds of CG 31, myrcene [01.008], d-limonene [01.045], 1-isopropyl-4-benzene [01.002] and β-caryophyllene [01.007] were applied as group NOAELs to the subassessment group II, III, IVe and V respectively (EFSA CEF Panel, 2015c,d).

For the remaining compounds[28] toxicity studies performed with the compounds under assessment and NOAEL values derived from toxicity studies were not available and read-across was not possible. Therefore, the threshold of toxicological concern (TTC) approach was applied (EFSA FEEDAP Panel, 2017b).

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26 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.

27 Terpineol is a mixture of four isomers: α-terpineol [02.014], a mixture of (R)-(+)–α-terpineol and (S)-(−)–α-terpineol, β-terpineol, γ-terpineol and 4-terpinenol [02.072] (or δ-terpineol). The specification for terpineol [02.230] covers α, β, γ and δ-terpineol. Composition of mixture: 55–75% α-terpineol, 16–23% γ-terpineol, 1–10% cis-β-terpineol, 1–13% trans-β-terpineol and 0–1% δ-terpineol (EFSA CEF Panel, 2015c) FGE.18Rev 3.

28 Trans-Murol-5-en-4-ol, alismol, δ-cadinol, trans-sabinene hydrate, cis-sabinene hydrate, δ-elemene, β-elemene, 3,7,10-humulatriene, germacr-1(10),4(14),5-triene.
As the result of the hazard characterisation, a reference point was identified for each component in the assessment group based on the toxicity data available (NOAEL from in vivo toxicity study or read across) or from the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class (i.e. 3, 0.91 and 0.15 mg/kg bw per day, respectively, for Cramer Class I, II and III compounds) (Table 5).

For risk characterisation, the margin of exposure (MOE) was calculated for each component as the ratio between the reference point and the exposure. For each assessment group, the combined (total) margin of exposure (MOET) was calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual substances (EFSA SC, 2019). An MOET > 100 allowed for interspecies- and intra-individual variability (as in the default 10 × 10 uncertainty factor). The compounds resulting individually in an MOE > 50,000, listed in the footnote, were not further considered in the assessment group as their contribution to the MOE(T) is negligible.29

The approach to the safety assessment of black pepper oil for the target species is summarised in Table 5.

Table 5: Compositional data, intake values (calculated for chickens for fattening at 5.0 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of black pepper oil classified according to assessment groups

| Assessment group | Essential oil composition | Exposure | Hazard characterisation | Risk characterisation |
|------------------|---------------------------|----------|------------------------|----------------------|
|                  | FLAVIS No | Highest conc. in the oil | Highest Feed conc. | Intake(a) | Cramer Class(b) | NOAEL(c) | MOE | MOET |
| Constituent      | – | % | mg/kg | mg/kg bw/day | – | mg/kg bw/day | – | – |
| CG 6             | Linalool | 0.0213 | 0.54 | 0.027 | 0.002 | (I) | 117 | 48,630 |
|                  | trans-Muurol-5-en-4-α-ol | – | 0.35 | 0.018 | 0.002 | III | 3 | 1,888 |
|                  | Alismol | – | 0.24 | 0.012 | 0.001 | III | 3 | 2,808 |
|                  | d-Cadinol | – | 0.24 | 0.012 | 0.001 | III | 3 | 2,796 |
|                  | MOET CG 6 | – | – | – | – | – | – | 780 |
| CG 31, II (Acyclic alkanes) | Myrcene | 0.008 | 2.17 | 0.11 | 0.01 | (I) | 44 | 4,521 |
|                  | trans-3,7-Dimethyl-1,3,6-octatriene | – | 0.27 | 0.013 | 0.001 | I | 44 | 36,172 |
|                  | MOET CG 31, II | – | – | – | – | – | – | 4,019 |
| CG 31, III (Cyclohexene hydrocarbons) | α-Phellandrene | 0.0106 | 2.18 | 0.11 | 0.01 | (I) | 250 | 25,525 |
|                  | Limonene | 0.001 | 13.37 | 0.67 | 0.06 | (I) | 250 | 4,167 |
|                  | β-Phellandrene | 0.055 | 2.14 | 0.11 | 0.01 | (I) | 250 | 26,038 |
|                  | δ-Elemene | 0.039 | 3.17 | 0.16 | 0.01 | I | 3 | 210 |
|                  | β-Elemene | – | 0.53 | 0.03 | 0.002 | I | 3 | 1,273 |
|                  | β-Bisabolene | 0.028 | 1.44 | 0.07 | 0.006 | (I) | 250 | 38,704 |
|                  | MOET CG 31, III | – | – | – | – | – | – | 170 |
| CG 31, IV (Bi-, tricyclic, non-aromatic hydrocarbons) | 1-Isopropyl-4-methylbenzene | 0.002 | 0.75 | 0.038 | 0.003 | (I) | 154 | 45,684 |
|                  | MOET CG 31, V | – | – | – | – | – | – | 45,684 |

29 Compounds included in the assessment groups but not reported in the table: 4-terpinenol, α-terpineol, cis-sabinene hydrate, 1,8-cineole, trans-β-farnesene, alpha-terpinene, γ-terpinene, isoterpinolene, terpinolene, camphene, α-cubebene, cyclosativene, β-cubenene, bicyclogermacrene, β-selinene, α-muurolene, α-selinene, α-gurjunene, β-copaene.
As shown in Table 5, for all the assessment groups and individual constituents, the MOE(T) was ≥ 170. Therefore, no safety concern was identified for the oil under assessment when used as a feed additive for chickens for fattening at the proposed use level (5 mg/kg). From the lowest MOET of 170 (for CG 31, III) in chickens for fattening, the MOET was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 6.

As shown in Table 5, for all the assessment groups and individual constituents, the MOE(T) was ≥ 170. Therefore, no safety concern was identified for the oil under assessment when used as a feed additive for chickens for fattening at the proposed use level (5 mg/kg). From the lowest MOET of 170 (for CG 31, III) in chickens for fattening, the MOET was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 6.

**Table 6:** Combined margin of exposure (MOET) for the assessment group CG 31, III (cyclohexene hydrocarbons) calculated for the target species at the proposed use level

| Body weight (kg) | Feed intake (g DM/day) | Proposed use level (mg/kg feed) | Lowest MOET |
|------------------|------------------------|---------------------------------|-------------|
| Chickens for fattening | 2 | 158 | 5 | 170 |
| Laying hens | 2 | 106 | 8 | 252 |
| Turkeys for fattening | 3 | 176 | 7 | 226 |
| Piglets | 20 | 880 | 9.5 | 303 |
| Pigs for fattening | 60 | 2,200 | 11.5 | 361 |
Table 6 shows that for all species, the MOET exceeds the value of 100. Because glucuronidation is an important metabolic reaction to facilitate the excretion of the components of the essential oil, the use of black pepper oil as additive in cat feed needs a wider margin of exposure. Considering that cats have an unusually low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021), an MOET of 500 is considered adequate.

No specific proposals have been made by the applicant for the use level in water for drinking. The Panel considers that the use of the additive in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed (EFSA FEEDAP Panel, 2010).

Conclusions on safety for the target species

The FEEDAP Panel concludes that black pepper oil is safe up to the maximum proposed use levels in complete feed of 5 mg/kg for chickens for fattening and other minor growing poultry, 8 mg/kg for laying hens and other laying/breeding birds kept for egg production/reproduction, 7 mg/kg for turkeys for fattening, 9.5 mg/kg for piglets and other minor Suidae, 11.5 mg/kg for pigs for fattening, 14 mg/kg for sows and dairy cows and minor dairy ruminants (other than sheep/goats), 8.5 mg/kg in rabbits and 20 mg/kg in veal calves, cattle for fattening and other growing ruminants, sheep/goats, horses, salmonids and other fin fish, dogs, cats and ornamental fish. For all the other species, the additive is considered safe at 5 mg/kg complete feed.

The FEEDAP Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.2.2.2. Safety for the consumer

Black pepper oil is added to a wide range of food categories for flavouring purposes. Although individual consumption figures are not available, the Fenaroli’s handbook of flavour ingredients (Burdock, 2009) cites values of 7,104 μg/kg bw per day (FEMA 3119). Fenaroli’s also reports use levels in food and beverages in the range of 3 mg/kg up to 206.4 mg/kg (Burdock, 2009).

The majority of the individual constituents of the essential oil under assessment are currently authorised as food flavourings without limitations and have been already assessed for consumer safety when used as feed additives in animal production (see Table 1, Section 2.1).

No data on residues in products of animal origin were made available for any of the constituents of the essential oil. However, the Panel recognises that the constituents of black pepper oil are expected to be extensively metabolised and excreted in the target species.

Considering the above and the reported human exposure due to direct use of black pepper oil in food (Burdock, 2009), it is unlikely that the consumption of products from animals given black pepper oil at the proposed maximum use level would increase human background exposure. Consequently, no safety concern would be expected for the consumer from the use of black pepper oil up to the highest safe use level in feed for the target animals.
3.2.2.3. Safety for user

No specific data were provided by the applicant regarding the safety of the additive for users. The applicant provided some papers retrieved from a literature search. None of the papers provided were considered relevant for the risk assessment due to some limitations (some studies reported beneficial effects only, the test item used was not sufficiently characterised).

The applicant produced a safety data sheet30 for black pepper oil where hazards for users have been identified. The essential oil under assessment should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitisers.

3.2.2.4. Safety for the environment

*P. nigrum* is not native to Europe. Therefore, the safety for the environment is assessed based on the individual components of the essential oil.

The major components ([β]-caryophyllene, limonene, sabinene and α-pinene) and 13 additional components (β-pinene, 6,3-carene, myrcene, α-phellandrene, p-cymene, terpinolene, 4-terpinenol, linalool, camphene, γ-terpinene, α-terpinene, α-terpineol and 1,8-cineole) accounting together for more than 78% of the composition of the oil have been evaluated by EFSA as sensory additives for animal feed and they were considered to be safe for the environment at individual use levels higher than those resulting from the use of the essential oil in feed (see Table 1, section 1.2).

The remaining identified constituents of the essential oil are aliphatic mono- or sesquiterpenes partially substituted with functional groups. They are structurally related to the substances evaluated by EFSA as CG 6 (trans-murol-5-en-4-α-ol, alismol and δ-cadinol), CG 8 (cis- and trans-sabinene hydrate) and CG 31 (α-copaene, α-thujene, bicyclogermacrene, β-elemene, α-cubebene, β-selinene, trans-3,7-dimethyl-1,3,6-octatriene, cyclosativene, isoterpinolene, α-selinene, β-copaene, trans-β-farnesene and α-gurjunene) for use in animal feed (EFSA FEEDAP Panel, 2012a, 2015, 2016b,c) for which EFSA concluded that they will be ‘extensively metabolised by the target species and excreted as innocuous metabolites or carbon dioxide’ (see Section 3.2.2). Average feed levels of constituents of the essential oil are much lower than the use levels for substances belonging to CG 6, 8 and 31.

The applicant also provided data on the natural occurrence of 4-terpinenol, δ-elemene, 3,7,10-humulatriene and germacr-1(10),4(14),5-triene and β-caryophyllene epoxide, which showed that these compounds are occurring in plants present in Europe at concentrations higher than the levels resulting from the use of black pepper oil.5

Therefore, the use of the additive under the proposed conditions in animal feed is not expected to pose a risk for the environment.

3.3. Supercritical extract of black pepper

3.3.1. Characterisation

The supercritical extract under assessment is a colourless or light coloured (yellow, green, blue) clear mobile liquid with a spicy and woody odour. In 10 batches of the additive (originating from Sri Lanka), the mean specific gravity was between 0.873 and 0.879, the refractive index between 1.480 and 1.483 (specification: 1.475–1.490) and the specific optical rotation (four batches) between −10.36 and −13.79 (specification: −17 and −8).

Non-volatile components

Supercritical extract of black pepper is specified to contain a non-volatile fraction ≤10%. Analysis of three batches showed compliance with the specification (7.25% on average, range 7.0–7.5%). This fraction was shown to consist mostly of lipids (ca. 6.25% of the additive, average in three batches). The rest of the non-volatile fraction (some 1% of the extract) represents a minor fraction which consists of polysaccharides, flavonoids and/or amides at low concentration which are not expected to be of concern.31

30 Technical dossier/Supplementary Information (May 2021)/Annex XII_SIn reply_pepper_oil_black_steam_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1).

31 Technical dossier/Supplementary information May (2021)/Section II.
Volatile components

The product specifications for the volatile components are based on the standards developed by the International Organisation for Standardization (ISO) 3,061:2008 for black pepper oils, which were adapted to reflect the concentrations of the main volatile components, analysed by GC-FID and expressed as %GC area. These components are β-caryophyllene, limonene, sabinene and α-pinene. Analysis of five batches for each product analysed by GC-FID showed compliance with these specifications. When analysed by GC-MS, these four compounds account for 49.2% on average (range 39.6–54.4%) of the % GC area (Table 7).

Table 7: Major volatile constituents of supercritical extract from the fruits of *Piper nigrum* L. as defined based on ISO standard (3,061:2008): specifications and batch-to-batch variation based on the analysis of five batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

| Constituent            | EU register name | CAS No | FLAVIS No | % GC area supercritical extract of black pepper | Specification | Mean | Range     |
|------------------------|------------------|--------|-----------|-----------------------------------------------|---------------|------|-----------|
|                        |                  |        |           |                                               |               |      |           |
| β-Caryophyllene        |                  | 87-44-5| 01.007    | 8.0–30.0                                      | 15.1          |      | 13.9–17.2 |
| Limonene               |                  | 138-86-3| 01.001   | 10.0–18.0                                     | 12.2          |      | 11.9–12.5 |
| Sabinene (4(10)-thujene) |                | 3387-41-5| 01.059 | 5.0–17.0                                      | 10.6          |      | 6.2–12.1  |
| α-Pinene (pin-2(3)-ene)|                  | 80-56-8| 01.004    | 7.0–18.0                                      | 11.4          |      | 8.0–12.6  |
| Total                  |                  |        |           |                                               | 49.2          |      | 39.6–54.4 |

The applicant provided the full characterisation of the volatile and non-volatile fractions of the five batches of the supercritical extract of black pepper by GC-MS and GC-FID. In total, 56 peaks were identified and accounted on average for 92.2% (91.8–92.4%) of the % GC area. Besides the four major compounds indicated in the product specification, 41 other compounds were detected at the individual levels > 0.1% (Table 8). Together these 45 compounds accounted for 91.6% (91.2–91.9%) of the % GC area. The remaining 11 compounds accounting for 0.63% are listed in the footnote. When related to the whole additive as 100%, these figures may be reduced of about 6.25–10% to account for the presence of other non-volatiles in the supercritical extract.

Table 8: Characterisation of the constituents of the volatile and the non-volatile fractions of supercritical extract of black pepper (*Piper nigrum* L.) accounting for > 0.1% of the composition not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

| Constituent                  | EU register name | CAS No | FLAVIS No | % GC area Supercritical extract of black pepper | Mean(a) | Range    |
|------------------------------|------------------|--------|-----------|-----------------------------------------------|---------|----------|
| **Volatile fraction**        |                  |        |           |                                               |         |          |
| β-Pinene (pin-2(10)-ene)    |                  | 127-91-3| 01.003    | 8.75                                          | 8.37–8.99|          |
| δ-3-Carene                  |                  | 13466-78-9| 01.029 | 8.98                                          | 7.57–13.88|          |
| α-Copaene                   |                  | 3856-25-5| –         | 2.84                                          | 2.59–3.07|          |
| δ-Elemene                   |                  | 20307-84-0| 0.039   | 1.26                                          | 1.10–1.74|          |
| Myrcene                     |                  | 123-35-3| 0.008     | 2.22                                          | 2.15–2.29|          |

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32 Technical dossier/Supplementary information May (2021)/Annex VII Pepper oil black ISO
33 Technical dossier/Supplementary information May (2021)/Annex_III_SIn_Reply Pepper oil black chromatography.
34 Constituents in black pepper oil supercritical extract between <0.1 and ≥0.05%: α-Terpinol; 4-Hydroxy-4-methylpentan-2-one; Piperonal; cis-3,7-Dimethyl-1,3,6-octatriene; trans-beta-Farnesene; α-Terpinene; 1-(Piperidin-1-yl)dodecan-1-one; Piperidine; 1-1-oxo-3-phenyl-2-propenyl)-, (E,E)-1-(oxo-2,4-decadienyl) pyrrolidine; 1-(Piperidin-1-yl)dodecan-1-one between -0.05% and -0.01%; phytol; Piperidine, 1-1-oxo-3-phenyl-2-propenyl)-, 1-(Piperidin-1-yl)dodecan-1-one, 4-Hydroxy-4-methylpentan-2-one.
Beside piperanine detected by GC–MS (Table 8), the presence of aromatic amides (e.g. piperine, piperlonguminine) in pepper fruits is also mentioned in the Commentary to the European Pharmacopoeia (EUPh Commentary, 2020). The applicant performed a quantitative analysis by HPLC–UV of piperine in five batches (range: 0.5–2.0%)\(^{16}\) and in additional six batches (range: 0.71–2.14%)\(^{22}\) and of piperlonguminine, measured using HPLC with MS in four batches (range: < 0.001–0.003%).\(^{35}\)

The occurrence of safrole (not quantified) is also reported in the EFSA compendium based on one reference (Wrba et al., 1992). However, safrole was not identified in any analysed batches of supercritical extract of black pepper (LOD 3 mg/kg).

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35 Technical dossier/Supplementary information (May 2021)/Annex V.
3.3.1.1. Impurities

The applicant provided analyses on three batches of supercritcial extract of black pepper investigating possible presence of impurities. These analyses included mercury (0.005–0.011 mg/kg), cadmium (0.005 mg/kg), lead (<0.05 mg/kg), pesticide residues, mycotoxins (sum of fumonisins B1 and B2 < 40 μg/kg, aflatoxins B1, B2, G1 and G2 < 1 μg/kg, ochratoxin A < 2 μg/kg), dioxins and dioxin-like PCBs36. Microbial contamination was not investigated as the extract contains no water. The levels of dioxins and the sum of dioxins and dioxin-like-PCBs ranged from 1.26 to 1.37 ng WHO-PCDD/F-TEQ/kg and from 0.6 to 2.1 ng WHO-PCDD/F-PCB-TEQ/kg, respectively. The non-dioxin-like PCBs ranged from 1.2 to 3.1 ng/kg additive.

The detected amounts of the above-described undesirable substances do not raise safety concerns.

3.3.1.2. Shelf-life

The typical shelf-life of the additive is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light). However, no data were provided to support this statement.

3.3.1.3. Conditions of use

Supercritical extract of black pepper is intended to be added in feed for dogs and cats at a level of 1.5 mg/kg complete feed.

3.3.2. Safety

The assessment of the safety of supercritical extract of black pepper is based on the maximum use level proposed by the applicant (Section 3.3.1.3).

Many of the components of the mixture, accounting for about 82% of the % GC peak areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food, without limitations and for use in feed at individual levels higher than those resulted from the intended use of the essential oil in feed. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Additional considerations on the volatile components (accounting for 8.5%) not assessed by EFSA have been addressed in Section 3.2.2.

Besides the components already identified in black pepper oil, seven additional constituents were detected in the supercritical extract, namely 1-(piperidin-1-yl)decan-1-one, 1-(1-oxo-3-phenyl-2-propenyl) piperidine, (E,E)-1-(oxo-2,4-decadienyl) pyrrolidine, (2E,4E)-1-(piperidin-1-yl)deca-2,4-dien-1-one, 1-(piperidin-1-yl)dodecan-1-one, (2E,4E)-1-(piperidin-1-yl)dodeca-2,4-dien-1-one and piperanine. Aliphatic and arylalkyl amines and amides have been evaluated by EFSA (EFSA CEF, 2015b), which concluded on the absence of genotoxic potential, based on negative results for 15 derivatives, including piperine [14.003], piperidine [14.010] and deca-(2E,4E)-dienoic acid isobutylamide [16.091]. This conclusion is extended to the piperine derivatives present in the additive.

3.3.2.1. Safety for the target species

Tolerance studies and/or toxicological studies made with the supercritical extract of black pepper under application were not submitted.

In view of the similarity in the composition of the volatile fraction of the supercritical extract of black pepper with that of the essential oil (see Sections 3.3.1.1 and 3.3.2.1), and considering the lower concentrations of volatiles in the supercritical extract and the lower use levels of supercritical extract in feed for cats and dogs (1.5 mg/kg for the supercritical extract compared to 20 mg/kg for the essential oil), the FEEDAP Panel considers that the same conclusions reached on the safety for cats and dogs for black pepper oil apply to the supercritical extract (see Section 3.2.2.3), except for piperine derivatives, for which a separate assessment is presented.

Toxicological data from subchronic studies, from which NOAEL values could be derived, were available for piperine [14.003] in CG 28 (EFSA FEEDAP Panel, 2016a) and deca-(2E,4E)-dienoic acid isobutyl-amide [16.091] (EFSA CEF Panel, 2015b).

In 2015, the CEP Panel evaluated a 90-day study with piperine [14.003] conducted in rats according to OECD Test Guideline (TG 408) (Bauter, 2013 as reported in EFSA CEF Panel, 2015b). Four groups of adult Crl: Sprague–Dawley® CD® IGS rats (10/sex per group) were fed with diets, calculated

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36 Technical dossier/Supplementary Information (October 2021)/Section II/2021-10-05-pepper-supercritical-impurities.pdf
to provide piperine intake levels of 4.8, 14.5 and 47.8 mg/kg bw per day in males and 4.8, 14.6 and 48.4 mg/kg bw per day in females, giving an average daily intake of 0 (vehicle), 5, 15 or 50 mg/kg bw per day for males and females. There were no mortalities. No clinical, ophthalmological, gross and microscopic changes or clinical pathology or organ weight changes were attributed to the administration of piperine. A statistically significant and dose-dependent increase in cholesterol was observed in males administered with piperine at 15 and 50 mg/kg bw per day. Based on the dose-dependent increase in plasma cholesterol levels in males the CEF Panel identified an NOAEL of 5 mg/kg bw per day (the lowest dose tested).

As concerns piperine and the piperine derivatives present in the preparation, the FEEDAP Panel considered to apply a component-based approach using the NOAEL of 5 mg/kg bw per day available for piperine (EFSA CEF Panel, 2015b) and structurally related compounds (Table 9).

The assessment of the safety of piperine derivatives present in supercritical extract of black pepper for dogs and cats are summarised in Table 9. The calculations were done at the proposed use level of 1.5 mg/kg complete feed.

Table 9: Compositional data, intake values (calculated for dogs and cats at 1.5 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of supercritical extract of black pepper belonging to the assessment group piperine derivatives

| Supercritical extract composition | Exposure | Hazard characterisation | Risk characterisation |
|----------------------------------|----------|------------------------|----------------------|
|                                  |          | Highest Feed conc. | Daily Intake | MOE | MOET | MOE | MOET |
| Assessment group                 |          | Dogs | Cats | Cramer Class | NOAEL(b) | Dogs | Cats |
| CG 30                            |          |       |       |               |           |       |       |
| Deca-(2E,4E)-dienoic acid isobutyl-amide | 0.308 | 0.0046 | 0.00009 | 0.00010 | (III) | 10 | 114,312 | 95,260 |
| Piperine derivatives             |          |       |       |               |           |       |       |
| 1-(Piperidin-1-yl) deca-1-one    | 0.033    | 0.0005 | 0.00001 | 0.00001 | (III) | 5 | 527,104 | 439,253 |
| 1-(1-oxo-3-phenyl-2-propenyl)-piperidine | 0.065 | 0.0010 | 0.00002 | 0.00002 | (III) | 5 | 271,082 | 225,902 |
| (E,E)-1-(oxo-2,4-decadienyl) pyrrolidine | 0.088 | 0.0013 | 0.00003 | 0.00003 | (III) | 5 | 199,745 | 166,454 |
| (2E,4E)-1-(Piperidin-1-yl)deca-2,4-dien-1-one | 0.338 | 0.0051 | 0.00010 | 0.00012 | (III) | 5 | 52,131 | 43,443 |
| 1-(Piperidin-1-yl)dodecan-1-one  | 0.104    | 0.0016 | 0.00003 | 0.00004 | (III) | 5 | 169,426 | 141,189 |
| (2E,4E)-1-(Piperidin-1-yl)dodeca-2,4-dien-1-one | 0.234 | 0.0035 | 0.00007 | 0.00008 | (III) | 5 | 75,300 | 62,750 |
| Piperine                         | 0.829    | 0.0124 | 0.00024 | 0.00028 | (III) | 5 | 21,225 | 17,688 |

37 1-(piperidin-1-yl)decan-1-one, 1-(1-oxo-3-phenyl-2-propenyl)-piperidine, (E,E)-1-(oxo-2,4-decadienyl) pyrrolidine, (2E,4E)-1-(piperidin-1-yl)deca-2,4-dien-1-one, 1-(piperidin-1-yl)dodecan-1-one, (2E,4E)-1-(piperidin-1-yl)dodeca-2,4-dien-1-one and piperanine.
The FEEDAP Panel concludes that the supercritical extract of black pepper is safe up to the maximum proposed use level in complete feed of 1.5 mg/kg for cats and dogs.

3.3.2.2. Safety for user

No specific data were provided by the applicant regarding the safety of the additive for users. The applicant provided some scientific publications retrieved from a literature search. None of the scientific publications provided were considered relevant for the risk assessment due to some limitations (some studies reported beneficial effects only, the test item used was not sufficiently characterised).

The applicant produced a safety data sheet for the supercritical extract of black pepper where hazards for users have been identified. The additive under assessment should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser.

3.4. Black pepper oleoresin

3.4.1. Characterisation

The additive is an olive green coloured viscous liquid, with characteristic aroma and pungency of black pepper. Black pepper oleoresin is identified with the CAS number 84929-41-9, the EINECS number 284-524-7, the FEMA number 2846 and the CoE number 347.

Black pepper oleoresin contains by specification, 20-50% piperine (selected as the phytochemical marker), 15-40% volatiles and < 0.003% residual solvents (ethyl acetate < 0.0019%, ethyl acetate/hexane < 0.0017% and hexane/acetone < 0.0025). Analyses of several batches of oleoresins of different origin (oleoresin A, B and C) and obtained by three different extraction processes from different producers, showed compliance with the proposed specification (Table 10).
Table 10: Proposed specifications for black pepper oleoresin and batch-to-batch variation for the different products. The results are expressed as % (w/w)

| Specification          | Oleoresin A       | Oleoresin B       | Oleoresin C       |
|------------------------|-------------------|-------------------|-------------------|
| %                      | Range (%)         | Range (%)         | Range (%)         |
| Piperine\(^{(a)}\)    | 20-50             | 40.0-40.4         | 39.2-39.7         | 29.9-31.0         |
| Volatiles\(^{(b)}\)   | 15-40             | 20-28             | 29.1-32.4         | 33.4-36.6         |
| Residual solvents     | < 0.003           | 0.0015-0.0019     | 0.0016-0.0017     | < 0.0025          |

\(^{(a)}\): Determined by spectrophotometry in oleoresin A (3 batches) and by HPLC in oleoresins B and C (2 batches each).
\(^{(b)}\): Volatile part covers all the volatile ingredients (i.e. including the essential oil and humidity) is determined as loss on drying and does not include piperine.

Table 11 summarises the results of the proximate analysis of black pepper oleoresins (seven batches from three different producers). For oleoresins A and C, the content of essential oil was also determined by distillation, giving consistent results with those reported for volatiles in the proximate analysis.

Table 11: Proximate analysis of black pepper oleoresin (Piper nigrum L.) based on the analysis of seven batches for the three products originating from different manufacturing processes. The results are given as ranges and are expressed as % (w/w)

| Proximate analysis | Range % (w/w) | Oleoresin A\(^{(a)}\) | Oleoresin B\(^{(b)}\) | Oleoresin C\(^{(b)}\) |
|--------------------|---------------|-----------------------|-----------------------|-----------------------|
| Humidity + volatiles | Loss on drying | 20-28                 | 29.1-32.4             | 33.4-36.6             |
| Water              | Titration     | –                     | 0.14-0.36             | –                     |
| Dry matter         | 100 – (loss on drying) | 72-80                 | 67.6-70.9             | 63.4-66.6             |
| Ash                | Gravimetry    | < 0.1                 | < 0.1                 | < 0.1                 |
| Lipids             | Gravimetry    | 13.3-18.7             | 1.24-3.87             | 19.5-20.8             |
| Protein (adjusted) | Kjeldahl      | 5.24-5.74             | 8.72-11.5             | 9.39-9.54             |
| Fibre              | Gravimetry    | 0.3-0.4               | 0.34-0.60             | 0.19-0.23             |
| Lipids + protein + fibre\(^{(c)}\) | – | 19.1-24.8             | 13.1-13.2             | 29.1-29.2             |

\(^{(a)}\): 3 batches.
\(^{(b)}\): 2 batches.
\(^{(c)}\): calculated for the individual batches.

The applicant provided the full characterisation (volatile and non-volatile fractions) of the four batches of black pepper oleoresin A obtained by GC-MS.\(^{40}\) The constituents identified were 96 (plus 2 unidentified), 53 of which were detected at individual levels > 0.1%.

Table 12 summarises the results of the characterisation of the volatile and non-volatile fractions of Oleoresin A by GC-MS considering the constituents accounting for > 0.1% (53 compounds). The remaining 43 compounds (ranging between 0.01% and 0.09% and accounting together for 0.94–1.68% of the oleoresin) are listed in the footnote.\(^{41}\) In the same batches, the levels of propylene glycol, triacetin and glyceryl monoacetate used as carriers during the manufacturing process and analysed by GC-MS were 2.84% for propylene glycol (range 0.236–6.12%), 2.89% for triacetin (range 0.039–6.36%) and 0.23% (0.005–0.37%) for glyceryl monoacetate. Piperine determined by GC-MS was the major component detected (21.7–40.4% of Oleoresin A). The GC results for piperine differ from those reported in Table 10 for the same batches of oleoresin A (40–40.4%) which were obtained using spectrophotometry and were considered more reliable for quantitative purposes of piperine.

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\(^{40}\) Technical dossier/Supplementary information (January 2022)/Annex_1_SI, Reply Pepper oleoresin black, full_char_J9_conf.

\(^{41}\) For oleoresin A (43 compounds): 1,2-propanediol 1-acetate, propyl isovalerate, hexadecanoic acid, phytol, 4-terpinenol, \(\alpha\)-terpineol, cubeol, T-cadinol, \(\alpha\)-phellandren-8-ol, 2-(4-methylphenyl)propan-2-ol, \(\gamma\)-terpinol, \(\gamma\)-terpinyl acetate, \((-\rightarrow\)\(\alpha\)-elemol, nerolidol, cis-sabinene hydrate, (Z)-sabinol, eucarvone, butane-2,3-diol, curzerene, 1,8-cineole, (E,E)-N-isobutyl-2,4-hexadecadienamide, (2E,4E)-N-isobutyrltetradeca-2,4-dienamide, piperonal, methyl piperate, cis-3,7,13-trimethyl-1,3,6-octatriene, trans-3,7-dimethyl-1,3,6-octatriene, \(\alpha\)-terpinolene, \(\beta\)-phellandrene, \(\alpha\)-zingiberene, isoterpinolene, (E,E)-\(\gamma\)-bisabolene, calamenene, camphene, \(\alpha\)-cubebene, \(\beta\)-cubebene, \(\beta\)-copaene, \(\gamma\)-murolene, bicyclogermacrene, isoaromadendrene epoxide, 1-piperidino carbazole, piperidine, 1-acetyl-(E,E)-1-(2,4-dodecadienoyl)-pyrrolidine and (2E,4E)-1-(piperidin-1-yl)dodeca-2,4-dien-1-one.
Table 12: Characterisation of the constituents of the volatile and the non-volatile fractions of black pepper oleoresin (*Piper nigrum* L.) accounting for > 0.1% of the composition based on the analysis of four batches of Oleoresin A (mean and range). The results are expressed as % (w/w) of black pepper oleoresin.

| Constituent                           | EU register name | CAS No     | FLAVIS No | Mean(a) | Range   |
|---------------------------------------|------------------|------------|-----------|---------|---------|
| **Volatile fraction**                 |                  |            |           |         |         |
| β-Caryophyllene                       |                  | 87-44-5    | 01.007    | 4.81    | 0.07-8.12 |
| Limonene                              |                  | 138-86-3   | 01.001    | 3.20    | 2.43-4.00 |
| δ-3-Carene                           |                  | 13466-78-9 | 01.029    | 2.79    | 1.94-3.49 |
| β-Pinene (pin-2(10)-ene)             |                  | 127-91-3   | 01.003    | 2.19    | 1.35-3.65 |
| α-Pinene (pin-2(3)-ene)              |                  | 80-56-8    | 01.004    | 1.98    | 1.12-3.63 |
| δ-Elemene                            |                  | 20307-84-0 | 01.039    | 1.96    | 0.66-4.14 |
| α-Gurjunene                          |                  | 489-40-7   | –         | 1.91    | 0.10-7.32 |
| Sabine (4(10)-thujene)               |                  | 3387-41-5  | 01.059    | 1.27    | 0.59-1.75 |
| β-Selinene                           |                  | 17066-67-0 | –         | 0.65    | 0.14-1.10 |
| α-Copaene                            |                  | 3856-25-5  | –         | 0.65    | 0.27-0.80 |
| α-Phellandrene                       |                  | 99-83-2    | 01.006    | 0.53    | 0.42-0.79 |
| 3,7,10-Humulatriene                  |                  | 6753-98-6  | 01.043    | 0.45    | 0.05-0.84 |
| α-Selinene                           |                  | 473-13-2   | –         | 0.47    | 0.05-0.88 |
| δ-Elemene                            |                  | 33880-83-0 | –         | 0.40    | 0.16-0.71 |
| Myrcene                              |                  | 123-35-3   | 01.008    | 0.39    | 0.26-0.49 |
| Spathulenol(c)                       |                  | 6750-60-3  | –         | 0.34    | 0.14-0.54 |
| trans-β-Farnesene                    |                  | 18794-84-8 | –         | 0.30    | 0.01-0.66 |
| β-Bisabolene                         |                  | 495-61-4   | 01.028    | 0.32    | 0.21-0.42 |
| β-Caryophyllene epoxide              |                  | 1139-30-6  | 16.043    | 0.29    | 0.13-0.42 |
| δ-Cadinol                            |                  | 29350-73-0 | 01.021    | 0.27    | 0.02-0.43 |
| Germacra-1(10),4(14),5-triene        |                  | 23986-74-5 | 01.042    | 0.22    | 0.05-0.40 |
| 1-Isopropyl-4-methylbenzene (p-cymene)|                  | 99-87-6    | 01.002    | 0.19    | 0.11-0.25 |
| α-Thujene                            |                  | 2667 05-2  | –         | 0.17    | 0.04-0.27 |
| δ-Cadinol                            |                  | 19435-97-3 | –         | 0.14    | 0.08-0.25 |
| Linalool                             |                  | 78-70-6    | 02.013    | 0.13    | 0.01-0.20 |
| Germacrene B(c)                      |                  | 15423-57-1 | –         | 0.43    | 0.43     |
| Cyclosativene                        |                  | 22469-52-9 | –         | 0.21    | 0.02-0.76 |
| Terpinolene                          |                  | 586-62-9   | 01.005    | 0.16    | 0.13-0.20 |
| γ-Terpinene                          |                  | 99-85-4    | 01.020    | 0.11    | 0.07-0.14 |
| 1(5),11-Guaiadiene(c)                |                  | 3691-12-1  | 01.023    | 0.07    | 0.01-0.17 |
| **Non-volatile fraction**            |                  |            |           |         |         |
| Piperine(b)                          |                  | 94-62-2    | 14.003    | 31.5    | 21.7-40.4 |
| Piperoleine B(c)                     |                  | 30505-89-6 | –         | 2.09    | 0.33-3.39 |
| Piperanine(b)                        |                  | 23512-46-1 | –         | 1.61    | 0.47-2.25 |
| Pipersintenamide(c)                  |                  | 147030-09-9 | –         | 1.34    | 0.45-2.00 |
| Piperettine(c)                       |                  | 583-34-6   | –         | 1.23    | 0.22-3.07 |
| Undefined isomers of piperine(c)     |                  | 94-62-2    | –         | 1.70    | 1.12-2.33 |
| Kusunokinin(c)                       |                  | 58311-20-9 | –         | 1.02    | 0.60-2.21 |
| Retroflectamide A(c)                 |                  | 94079-67-1 | –         | 0.81    | 0.55-0.99 |
| Tricholein(c)                        |                  | 62510-52-5 | –         | 0.72    | 0.26-0.97 |
| (E)-1-(Piperidin-1-yl)hexadec-2-en-1-one(c) |      | 147030-05-5 | –         | 0.63    | 0.11-1.71 |
| Piperlonguminine                     |                  | 5950-12-9  | –         | 0.47    | 0.01-0.95 |
| Cubebin(c)                           |                  | 18423-69-3 | –         | 0.43    | 0.28-0.66 |
| Guineensine(c)                       |                  | 55038-30-7 | –         | 0.40    | 0.31-0.51 |

Black pepper oils and oleoresin for all animal species
Overall, it is estimated that considering the identified volatile and non-volatile components (accounting for 75.6–80.9% of the oleoresin) and the results of proximate analysis (lipids+proteins+ fibre 19.1–24%), the Oleoresin A is considered fully characterised.

The applicant also provided the characterisation of the volatile fraction of Oleoresin B (two batches) by GC-MS. The constituents identified were 60 (plus 5 unidentified), 35 of which detected at individual levels > 0.1%.\(^4^2\)

The volatile components of Oleoresin A and B are quantitatively and qualitatively similar. The FEEDAP Panel considered that Oleoresin A is representative of Oleoresin B, as concern the components of the volatile fraction. Comparable compositional data for the volatile fraction of the Oleoresin C were not available.

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *Piper nigrum* L. and its preparations.\(^4^3\) Piperidine alkaloids (including piperine, piperidine, piperlonguminine) and safrole were identified as potential substances of toxicological concern in the EFSA compendium of botanicals (online version)\(^1^4\) and the BELFRIT list for the fruits of *Piper nigrum* L.

However, safrole was not identified in any batches analysed of black pepper oleoresin (LOD for batches of Oleoresin A: 50 mg/kg and for batches of Oleoresin B 10 mg/kg).

As concern the non-volatile components, the presence of aromatic amides (e.g. piperine, piparinerine, piperlonguminine) in pepper fruits is also mentioned in the Commentary to the European Pharmacopoeia (EUPh Commentary, 2020). The applicant performed a quantitative analysis by HPLC of piperlonguminine and piperidine on four batches of black pepper oleoresin: two from oleoresin B (piperlonguminine mean 0.326%, range 0.301–0.351%; piperidine mean 0.037%, range 0.033–0.042%) and two from oleoresin C (piperlonguminine mean 0.266%, range 0.265–0.267%; piperidine mean 0.041%, range 0.036–0.046%).

### 3.4.1.1. Impurities

Analytical data have been provided by the applicant on the presence of impurities (seven batches). Mercury ranged from < 0.005 to < 0.01 mg/kg, lead ranged from < 0.01 to 0.05 mg/kg and cadmium measured < 0.01 mg/kg in all batches tested, and arsenic ranged from < 0.01 to < 0.1 mg/kg.

The same batches were also analysed for microbiological contamination:\(^4^4\) *Salmonella* spp. was not detected in any of the batch analysed. Enterobacteriaceae, aerobic plate count, moulds and yeasts were < 10 colony forming unit (CFU)/mL for all the batches tested.

Four batches out of the seven above were also analysed for the presence of pesticide residues: cypermethrin was found in two batches only (ranging from < 0.2 to 0.28 mg/kg), chlorpyriphos(–ethyl) and permethrin were detected in a single batch only (0.28 mg/kg and 0.2 mg/kg, respectively) and other screened pesticides were all below the LOQ. Mycotoxins were also measured:\(^4^5\) aflatoxin B1 ranged from 0.2 to 1.1 μg/kg; aflatoxin B2, G1 and G2 ranged from < 0.1 to 1 μg/kg; total PCDD/PCB

| Constituent | CAS No | FLAVIS No | % oleoresin A Mean\(^a\) | Range |
|-------------|--------|-----------|------------------------|-------|
| Dehydro{pipernonaline\(^c\)} | 107584-38-3 | – | 0.37 | 0.32–0.45 |
| Deca-(2E,4E)-dieneic acid isobutyl-amide\(^c\) | 18836-52-7 | – | 0.32 | 0.02–0.66 |
| (2E,4E, 10E)-N-Isobutylhexadeca-2,4,10-trienamide\(^c\) | 5422-81-1 | – | 0.31 | 0.00–0.72 |
| γ-Sitosterol\(^c\) | 83-47-6 | – | 0.23 | 0.16–0.30 |
| Isospathulenol\(^c\) | 88395-46-4 | – | 0.18 | 0.05–0.55 |
| 1-(1-oxo-3-phenyl-2-propenyl)-piperidine\(^b\) | 5422-81-1 | – | 0.18 | 0.09–0.36 |
| (2E,4E)-1-(Piperidin-1-yl)deca-2,4-dien-1-one\(^b\) | – | – | 0.11 | 0.07–0.14 |
| Total | | | 70.7 | 67.6–74 |

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

\(^a\): Mean calculated on four batches of oleoresin A.

\(^b\): Compound also detected in the supercritical extract.

\(^c\): Compound detected only in the oleoresin.

**Overall**, it is estimated that considering the identified volatile and non-volatile components (accounting for 75.6–80.9% of the oleoresin) and the results of proximate analysis (lipids+proteins+ fibre 19.1–24%), the Oleoresin A is considered fully characterised.

The applicant also provided the characterisation of the volatile fraction of Oleoresin B (two batches) by GC-MS. The constituents identified were 60 (plus 5 unidentified), 35 of which detected at individual levels > 0.1%.\(^4^2\)

The volatile components of Oleoresin A and B are quantitatively and qualitatively similar. The FEEDAP Panel considered that Oleoresin A is representative of Oleoresin B, as concern the components of the volatile fraction. Comparable compositional data for the volatile fraction of the Oleoresin C were not available.

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *Piper nigrum* L. and its preparations.\(^4^3\) Piperidine alkaloids (including piperine, piperidine, piperlonguminine) and safrole were identified as potential substances of toxicological concern in the EFSA compendium of botanicals (online version)\(^1^4\) and the BELFRIT list for the fruits of *Piper nigrum* L.

However, safrole was not identified in any batches analysed of black pepper oleoresin (LOD for batches of Oleoresin A: 50 mg/kg and for batches of Oleoresin B 10 mg/kg).

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Analytical data have been provided by the applicant on the presence of impurities (seven batches). Mercury ranged from < 0.005 to < 0.01 mg/kg, lead ranged from < 0.01 to 0.05 mg/kg and cadmium measured < 0.01 mg/kg in all batches tested, and arsenic ranged from < 0.01 to < 0.1 mg/kg.

The same batches were also analysed for microbiological contamination:\(^4^4\) *Salmonella* spp. was not detected in any of the batch analysed. Enterobacteriaceae, aerobic plate count, moulds and yeasts were < 10 colony forming unit (CFU)/mL for all the batches tested.

Four batches out of the seven above were also analysed for the presence of pesticide residues: cypermethrin was found in two batches only (ranging from < 0.2 to 0.28 mg/kg), chlorpyriphos(–ethyl) and permethrin were detected in a single batch only (0.28 mg/kg and 0.2 mg/kg, respectively) and other screened pesticides were all below the LOQ. Mycotoxins were also measured:\(^4^5\) aflatoxin B1 ranged from 0.2 to 1.1 μg/kg; aflatoxin B2, G1 and G2 ranged from < 0.1 to 1 μg/kg; total PCDD/PCB

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\(^{4^2}\) Technical dossier/Supplementary information (January 2022)/Annex_II_SIn_Reply Pepper oleoresin black_full_char_K10_O14.

\(^{4^3}\) Technical dossier/Supplementary information/Literature search.

\(^{4^4}\) Technical dossier/Supplementary information (January 2022)/Annex_III and Annex_IV.

\(^{4^5}\) Technical dossier/Supplementary information (January 2022)/Annex_IV.
TEQ\textsuperscript{15} were on average for lower bound 0.75 pg/L, range: 0.447–1.02 pg/L; for medium bound 0.87 pg/L, range: 0.577–1.14 pg/L; for upper bound 0.99 pg/L, range: 0.707–1.26 pg/L.

Results from analysis on possible solvent residues are reported in Section 3.4.1. The levels of residual solvents (ethyl acetate, hexane and acetone) were < 30 mg/kg, below the corresponding thresholds for class 2 (290 mg/kg for hexane) and class 3 (ethyl acetate and acetone) proposed by International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (EMA, 2010).

The detected amounts of the above-described undesirable substances do not raise safety concerns.

3.4.1.2. Shelf-life

The typical shelf-life of the additive is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light).\textsuperscript{23} However, no data supporting this statement were provided.

3.4.1.3. Conditions of use

Black pepper oleoresin is intended to be added to feed and water for drinking for all animal species without a withdrawal time. The applicant is proposing the levels as in Table 13. No use level has been proposed by the applicant for the use in water for drinking.

Table 13: Conditions of use for the oleoresin from the fruit of Piper nigrum L.: maximum proposed use levels in complete feed for the different target species

| Use level (mg/kg feed) | Chickens for fattening | Laying hens | Turkeys for fattening | Piglets | Pigs for fattening | Sows | Veal calves (milk replacer) | Cattle for fattening | Dairy cows | Sheep/goat | Horse | Rabbit | Salmons and other carnivorous fish\textsuperscript{(a)} | Dogs | Cats | Ornamental fish | Shrimps\textsuperscript{(b)} |
|------------------------|------------------------|-------------|-----------------------|---------|-------------------|------|--------------------------|---------------------|-----------|-----------|-------|--------|----------------------------------|-------|------|------------------------|-----------|
| 3.0                    |                        | 4.5         | 4.0                   | 5.5     | 6.5               | 7.5  | 12.5                     | 11.5                | 7.5       | 11.5      | 11.5  | 4.5    | Salmo salar (Atlantic salmon), Oncorhynchus kisutch (Coho salmon), Oncorhynchus mykiss (rainbow trout), Dicentrarchus labrax (European bass) and Sparus aurata (gilt-head bream). | 13.5   | 11.5 | 51.5                   | 17.5      |
|                         |                        |             |                       |         |                   |      |                          |                     |           |           |       |        | (b): including the family Penaeidae and Macrobrachium genus (e.g. Macrobrachium rosenbergii). |        |      |            |           |

\textsuperscript{(a)}: including Salmo salar (Atlantic salmon), Oncorhynchus kisutch (Coho salmon), Oncorhynchus mykiss (rainbow trout), Dicentrarchus labrax (European bass) and Sparus aurata (gilt-head bream).

\textsuperscript{(b)}: including the family Penaeidae and Macrobrachium genus (e.g. Macrobrachium rosenbergii).

3.4.2. Safety

The assessment of safety of black pepper oleoresin is based on the maximum use levels proposed by the applicant (Table 14).

Plant-derived constituents including ash, lipids, protein, fibres and other constituents which may occur in black pepper oleoresin are widely present in natural feeds and foods and are not considered as of safety concern. No concern is either expected for other constituent used as carriers such as propylene glycol and triacetin.
For the other 96 compounds in Oleoresin A, 3346 (representing on average 52.7% of black pepper oleoresin in the first four batches analysed by GC–MS) are authorised for use in food without limitations and for use in feed at individual use levels higher than those resulting from the intended use of the oleoresin in feed and are therefore not expected to be of safety concern. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

The rest of the 63 identified constituents, accounting for an average of 18% of black pepper oleoresin analysed by GC–MS, are substances not previously evaluated as feed or food flavourings. The majority of these constituents are structurally related to compounds authorised as food and/or feed flavourings as described below.

Additional considerations on the volatile components not assessed by EFSA have been addressed in Sections 3.2.2 and 3.3.2.

Additional compounds present in the oleoresin but not identified in the essential oil or in the supercritical extract (spathulenol, T-cadinol, T-muurolol, δ-cadinol, γ-terpineol, γ-terpinyl acetate, isopathulenol and cis-sabinene hydrate) are structurally similar to terpineol authorised as feed flavouring (EFSA FEEDAP Panel, 2012a).

Coniferyl alcohol is a metabolite of isoeugenol which is authorised as feed additive (EFSA FEEDAP Panel, 2012f). (E,E)-N-Isobutyl-2,4-hexadecadienamide, (E,E,4E,10E)-N-isobutylhexadeca-2,4,10-trienamide and (E,2E,4E)-N-isobutyltetradeca-2,4-dienamide are structurally similar to the authorised food flavouring deca-(2E,4E)-dienoic acid isobutyl-amide [16.091] (EFSA CEF Panel, 2015b). Piperlongumine, retrofractamide A and guineensine are structurally similar to food/feed flavourings piperonale (EFSA FEEDAP Panel, 2012c) and N-(1-propylbutyl)-1,3-benzodioxole-5-carboxamide (EFSA CEF Panel, 2014c). Isoaromadendrene epoxide is structurally related to the authorised feed flavouring β-caryophyllene epoxide (EFSA CEF Panel, 2014b).

α-Phellandren-8-ol, (Z)-sabinol, eucarvone and curzerene are structurally similar to feed flavouring additives allocated to CG 6, CG 8 and CG 14 which EFSA evaluated as safe for consumers (EFSA Journal, 2012a,d, 2016c).

Methyl piperate is structurally similar to piperonal, a metabolite of piperine which is an authorised feed additive, is considered safe for consumers. Cubebin and kusunokinin, as piperine and methyl piperate, are expected to be metabolised via the cleavage of methylenedioxy groups followed by glucuronidation and sulphation (EFSA FEEDAP Panel, 2016a).

Metabolic pathways for piperine in hepatocytes have been proposed, comprising: hydroxylation, ring opening, methylation, decarboxylation, reduction, glucuronidation and GSH conjugation. Piperine is bioactivated forming ortho-quinone and aldehyde intermediates, which were trapped by GSH (Li et al., 2020).

3.4.2.1. Toxicology

Genotoxicity

For fully defined mixtures, the EFSA Scientific Committee (EFSA SC) recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential (EFSA SC, 2019b).

1-(1-Oxo-3-phenyl-2-propenyl)-piperidine, 1-piperidine carboxaldehyde, 1-acetyl-, (E,E)-1-(2,4-dodecadienoyl)-pyrrolidine, (E,2E,4E)-1-(piperidin-1-yl)dodeca-2,4-dien-1-one, piperretine, piperanine, (E)-1-(piperidin-1-yl)hexadec-2-en-1-one, pipersintenamide, tricholein, pipereolene B and dehydropipernonaline are derivatives of piperine which EFSA concluded on the absence of genotoxicity potential (EFSA CEP Panel, 2015c).

No genotoxicity evaluation nor extrapolation has been done for alpha-phellandren-8-ol, (Z)-sabinol, eucarvone, curzerene, methyl piperate, cubebin and kusunokinin. The genotoxic potential of these substances was predicted using the QSAR Toolbox (V.4.4.1). In addition, to support the absence of genotoxic concerns for CG19 compounds and piperine derivatives, genotoxicity endpoints (gene mutation, chromosomal damage) of piperlongumine, 1-(1-oxo-3-phenyl-2-propenyl)-piperidine and

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46 Propyl isovalerate*; Hexadecanoic acid*; Phytol*; Linalool; 4-Terpinenol; alpha-Terpineol; Cubebol*; 2-(4-Methylphenyl) propan-2-ol*; (--)alpha-Elemol*; Nerolidol; Butane-2,3-diol*; 1,8-Cineole; Piperonal; Myrcene; cis,3,7,Dimethyl-1,3,6-octatriene*; alpha-Phellandrene; alpha-Terpinene; Limonene; gamma-Terpineene; Terpinolene; delta-Elemene; beta-Bisabolene; 1-Isopropyl-4-methylbenzene; Pin-2(3)-ene; Camphene; 4(10)-Thujene; Pin-2(10)-ene; delta-3-Carene; beta-Caryophyllene; beta-Caryophyllene epoxide; Deca-(2E,4E)-dienoic acid isobutyl-amide; piperine (isomer)*; piperine*. Some of these constituents are reported only in the first four batches analysed by GC–MS. These are marked with an asterisk.

47 Technical dossier/Supplementary information/Annex_10.
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(E,E)-1-(2,4-dodecadienoyl)-pyrrolidine were checked using the QSAR Toolbox (V.4.4.1). No alerts were found for alpha-phemlandren-8-ol and curzerene.

For (Z)-sabinol, eucarvone, methyl piperate, cubein, kusunokinin, piperlongumunine, 1-(1-oxo-3-phenyl-2-propenyl)-piperidine and (E,E)-1-(2,4-dodecadienoyl)-pyrrolidine, further evaluation was done by ‘read-across’ analyses of data available for similar substances to the target compounds.

For (Z)-sabinol, methyl piperate, cubein, kusunokinin and piperlongumunine and (E,E)-1-(2,4-dodecadienoyl)-pyrrolidine structural alerts were found however, the similar compounds were all predicted as negative.

For piperidine, 1-(1-oxo-3-phenyl-2-propenyl)-structural alerts were found. Nine categories of similar compounds were defined (two remained empty after subcategorisation). All the similar compounds were negative except for those in one category which were positive. However, in this category, limited number of similar compounds were identified (1 with S9 and 6 without S9).

Subchronic toxicity studies

Toxicological studies with the additive under assessment were not available. The applicant has provided a literature search. Most of the papers retrieved focused on beneficial effects of black pepper oils (e.g., antimicrobial, antioxidant, anticancer and cytotoxic potentials). The search identified a study that is considered relevant for the present assessment.

A black pepper oleoresin extract containing 45% piperine and 19.2% volatile oil was tested in a 56-day oral toxicity study in male rats (Bhat and Chandrasekhar, 1986) which received a diet (18% casein based) containing 0, 110, 220, 440 mg pepper oleoresin/kg feed (corresponding to 0, 5.5, 11, 22 mg/kg bw per day), 200 mg black pepper/kg feed (powdered and sieved) (corresponding to 10 mg/kg bw per day) or 100 mg piperine/kg feed (corresponding to 5 mg/kg bw per day). No significant differences among the different groups were observed for feed efficiency ratio, organ weight, haematology and blood chemistry parameters. Only the blood concentration of haemoglobin was significantly increased by the diet with 100 mg piperine/kg feed. Nitrogen retention and/or absorption was slightly (but significantly) increased in rats fed with 110 and 220 mg pepper oleoresin/kg feed and piperine (0.2%) compared to the control diet. Nitrogen retention was also slightly increased in rats fed with piperine (0.2%). Fat absorption was similar across all experimental groups. None of the results from this study suggest any adverse effects at the doses tested. The FEEDAP Panel notes that the study has major limitations, particularly the limited duration, the use of male rats only and the small number of animals tested and consequently lack of sensitivity of the study, which increase the uncertainty in the derivation of the NOAEL.

3.4.2.2. Safety for the target species

Considering the similarity in the composition of the volatile fraction of black pepper oleoresin and black pepper oil and the lower concentrations of volatiles in the black pepper oleoresin (20-36%) compared to the black pepper oil, the same conclusions reached on the safety for the target species for black pepper oil (see Section 3.2.2.3) apply to the intake of volatiles from black pepper oleoresin. Therefore, with respect to the exposure to the volatiles present in the additive, no safety concern is identified for black pepper oleoresin when used as a feed additive for all animal species up to the maximum proposed use levels in complete feed of 3 mg/kg for chickens for fattening, 4.5 mg/kg for laying hens and rabbits, 4 mg/kg for turkeys for fattening, 5.5 mg/kg for piglets, 6.5 mg/kg for pigs for fattening, 7.5 mg/kg for sows, 12.5 mg/kg in veal calves, 11.5 mg/kg for cattle for fattening, sheep, cats and horses, 13.05 mg/kg for salmon, 14 mg/kg for dogs, 51.5 mg/kg for ornamental fish and 17.5 mg/kg for shrimps.

As concerns piperine and the piperine derivatives present in the preparation, the FEEDAP Panel considered to apply a component-based approach using the NOAEL of 5 mg/kg bw per day.
available for piperine (EFSA FEEDAP Panel, 2015) also for the other piperine derivatives. For 1-acetylpiperidine and 1-formylpiperidine, the TTC approach has been applied (Table 14).

**Table 14:** Compositional data, intake values (calculated for chickens for fattening at 3.0 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of oleoresin of black pepper belonging to the assessment group piperine derivatives

| Supercritical extract composition | Exposure | Hazard characterisation | Risk characterisation |
|----------------------------------|----------|------------------------|----------------------|
| Assessment group                 | Highest conc. in the extract | Highest Feed conc. | Daily Intake | Cramer Class | NOAEL | MOE | MOET |
| Constituent                      | %        | mg/kg                  | mg/kg bw/day |                 | mg/kg bw/day |     |     |
| (2E,4E, 10E)-N-Isobutyloxadeca-2,4,10-trienamide | 0.72 | 0.022 | 0.0019 | (III) | 10 | 5,157 |
| Deca-(2E,4E)-dienoic acid isobutyl-amide | 0.66 | 0.020 | 0.0018 | (III) | 10 | 5,626 |
| **Piperine derivatives**         |          |                        |             |             |     |     | 2,691 |
| Piperine (GC–MS)                | 40.4     | 1.212                  | 0.1088      | (III) | 5 | 46   |
| Piperoleine B                    | 3.39     | 0.102                  | 0.0091      | (III) | 5 | 548  |
| Piperanine                       | 2.25     | 0.068                  | 0.0061      | (III) | 5 | 825  |
| Pipersintenamide                | 2.00     | 0.060                  | 0.0054      | (III) | 5 | 928  |
| Piperettine                      | 3.07     | 0.092                  | 0.0083      | (III) | 5 | 605  |
| (E,E)-piperine (isomer)         | 2.33     | 0.070                  | 0.0063      | (III) | 5 | 797  |
| Tricholein                      | 0.97     | 0.029                  | 0.0026      | (III) | 5 | 1,910 |
| (E)-1-(Piperidin-1-yl)hexadec-2-en-1-one | 1.71 | 0.051 | 0.0046 | (III) | 5 | 1,086 |
| Pipertongumine                  | 0.95     | 0.029                  | 0.0026      | (III) | 5 | 1,954 |
| Dehydropipernonaline            | 0.45     | 0.014                  | 0.0012      | (III) | 5 | 4,126 |
| Piperidine, 1-{(oxo-3-phenyl-2-propenyl)-} | 0.36 | 0.011 | 0.0010 | (III) | 5 | 5,186 |
| (E,E)-1-{(oxo-2,4-decadienyl) pyrrolidine} | 0.10 | 0.003 | 0.0003 | (III) | 5 | 18,025 |
| (2E,4E)-1-{(Piperidin-1-yl)deca-2,4-dien-1-one} | 0.14 | 0.004 | 0.0004 | (III) | 5 | 13,261 |
| Piperidine, 1-acetyl             | 0.01     | 0.000                  | 0.0000      | (III) | 0.15 | 6,962 |
| 1-Piperine carboxaldehyde       | 0.01     | 0.000                  | 0.0000      | (III) | 0.15 | 5,063 |
| MOET                            | 58.1%    |                        |             |             |     |     | 32   |

(a): Intake calculations for the individual components are based on the use level of 3 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

(b): When an NOAEL value is available or read-across is applied, the allocation to the Cramer class is put into parentheses.

(c): Values in **bold** refer to those components for which the NOAEL value was available, values in *italics* are the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class, other values (plain text) are NOAELs extrapolated by using read-across.

As shown in Table 14, for all the assessment groups and individual constituents, the MOE(T) was ≥ 32. From the lowest MOET of 32 (for piperine and piperdine derivatives group) in chickens for fattening, the MOET was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 15.
At the proposed use levels, the MOET was below the value of 100 for poultry species, pigs, dairy cows and rabbit. The maximum safe use levels in feed were calculated in order to ensure an MOET ≥ 100 for the different target species and > 500 for cats, considering their unusually low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021). The calculated maximum safe levels in feed are shown in Table 15.

No specific proposals have been made by the applicant for the use level in water for drinking. The Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

**Conclusions on safety for the target species**

The FEEDAP Panel concludes that the black pepper oleoresin is safe up to the maximum proposed use levels in complete feed of 12.5 mg/kg for veal calves, 11.5 for cattle for fattening and other minor growing ruminants, sheep/goats and horses, 14 mg/kg for dogs 13.5 for mg/kg for salmonids and other fish and 51.5 for ornamental fish. For the other species, the calculated safe concentrations in complete feed are 1 mg/kg for chickens for fattening and other minor growing poultry, 1.4 mg/kg for laying hens and other laying/breeding birds kept for egg production/reproduction, 1.3 for turkeys for fattening, 1.7 mg/kg for piglets and other minor growing Suidae, 2 mg/kg for pigs for fattening, 2.5 mg/kg for sows, 2.4 mg/kg for dairy cows and other dairy ruminants, 1.5 mg/kg for rabbits, 3.8 mg/kg for cats. For all the other species, the additive is considered safe at 1 mg/kg complete feed.

The FEEDAP Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

### 3.4.2.3. Safety for the consumer

Black pepper oleoresin is added to a wide range of food categories for flavouring purposes. Although individual consumption figures are not available, the Fenaroli’s handbook of flavour ingredients (Burdock, 2009) cites values of 0.2725 mg/kg bw per day (FEMA 2846). Fenaroli’s also reports use levels in food and beverages in the range of 1 mg/kg up to 1,600 mg/kg (Burdock, 2009).

The majority of the individual constituents of the oleoresin under assessment are currently authorised as food flavourings without limitations and have been already assessed for consumer safety when used as feed additives in animal production (see Table 1, Section 2.1).

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### Table 15: Combined margin of exposure (MOET) for the assessment group piperine derivatives calculated for the target species at the proposed use level and maximum safe use levels in feed calculated to ensure an MOET ≥ 100 (500 for cats)

| Body weight (kg) | Feed intake (g DM/day) | Proposed use level (mg/kg feed)(1) | Lowest MOET | Safe level (mg/kg feed) |
|------------------|------------------------|-----------------------------------|-------------|------------------------|
| Chickens for fattening | 2 | 158 | 3.0 | 32 | 1 |
| Laying hens | 2 | 106 | 4.5 | 48 | 1.4 |
| Turkeys for fattening | 3 | 176 | 4.0 | 43 | 1.3 |
| Piglets | 20 | 880 | 5.5 | 57 | 1.7 |
| Pigs for fattening | 60 | 2,200 | 6.5 | 68 | 2 |
| Sow lactating | 175 | 5,280 | 7.5 | 84 | 2.5 |
| Veal calves (milk replacer) | 100 | 1,890 | 12.5 | 133 | – |
| Cattle for fattening | 400 | 8,000 | 11.5 | 126 | – |
| Dairy cows | 650 | 20,000 | 7.5 | 81 | 2.4 |
| Sheep/goat | 60 | 1,200 | 11.5 | 126 | – |
| Horse | 400 | 8,000 | 11.5 | 126 | – |
| Rabbit | 2 | 100 | 4.5 | 50 | 1.5 |
| Salmon | 0.12 | 2.1 | 13.5 | 140 | – |
| Dogs | 15 | 250 | 14.0 | 148 | – |
| Cats | 3 | 60 | 11.5 | 126 | 3.8 |
| Ornamental fish | 0.012 | 0.054 | 51.5 | 504 | – |

(1): Complete feed containing 88% DM, milk replacer 94.5% DM.
No data on residues in products of animal origin were made available for any of the constituents of the oleoresin. However, the Panel recognises that the constituents of black pepper oleoresin are expected to be extensively metabolised and excreted in the target species. Therefore, a relevant increase of the uptake of the individual constituents by humans consuming products of animal origin is not expected.

Considering the reported human exposure due to direct use of black pepper oleoresin in food (Burdock, 2009), it is unlikely that the consumption of products from animals given black pepper oleoresin at the proposed maximum use level would increase human background exposure. Consequently, no safety concern would be expected for the consumer from the use of black pepper oleoresin up to the highest safe use level in feed for the target animals.

3.4.2.4. Safety for user

No specific data were provided by the applicant regarding the safety of the additive for users. The applicant provided some papers retrieved from a literature search. None of the papers provided were considered relevant for the risk assessment.

The applicant produced a safety data sheet57 for pepper oleoresin where hazards for users have been identified. The oleoresin under assessment should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser.

3.4.2.5. Safety for the environment

P. nigrum is not native to Europe. Therefore, the safety for the environment is assessed based on the individual components of the essential oil.

Most of the volatile components in the black pepper oleoresin have been evaluated as chemically defined feed flavourings by EFSA as sensory additives for animal feed; they were considered to be safe for the environment at individual use levels higher than those resulting from the use of the oleoresin in feed (see Table 1, Section 1.2).

Additional volatile components, accounting individually for less than 2% of the oleoresin and belonging to CG 6, 8 and 31, are structurally related to compounds already authorised as feed additives (see Section 3.4.2). These compounds are expected to be extensively metabolised and largely excreted as metabolites (EFSA FEEDAP Panel, 2012a, 2015, 2016b,c). Most of these compounds were also identified in black pepper oil.

At the proposed conditions of use in feed for food producing animals (up to 17.5 mg/kg in shrimps), all the volatile compounds not previously evaluated as chemically defined flavourings will result in concentrations in feed below 0.5 mg/kg complete feed, which is the dose below which the trigger value for the predicted environmental concentration (PEC_soil) of 10 μg/kg is not exceeded.

All the constituents present in the non-volatile fraction of pepper oleoresin oil (except piperine) have not been evaluated by EFSA with respect to the safety for the environment. These compounds account individually for < 2.1% of the oleoresin (see Table 13). At the proposed conditions of use in feed for food producing animals, they will result in concentrations in feed below the dose of 0.5 mg/kg complete feed.

Therefore, the FEEDAP Panel concludes that the use of pepper oleoresin as a flavour in animal feed is not expected to pose a risk for the environment.

3.5. Efficacy

The fruits of Piper nigrum and their preparations are listed in Fenaroli’s Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 2845 (black pepper oil) and 2,846 (black pepper oleoresin) and are recognised to flavour food.

Since their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

Black pepper oil, supercritical extract of black pepper and black pepper oleoresin from Piper nigrum may be produced from plants of different origins and by various processes resulting in preparations with different composition and toxicological profiles. Thus, the following conclusions apply only to black pepper oil, supercritical extract of black pepper and black pepper oleoresin.

57 Technical dossier/Supplementary Information (January 2022)/Annex_XL_SI_SIn reply_pepper_oleoresin_black. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1).
pepper oil and supercritical extract of black pepper which contain ≤ 3 mg/kg safrole and to black pepper oleoresin which contains ≤ 10 mg/kg safrole and are produced from the fruits of *Piper nigrum*.

The FEEDAP Panel concludes that black pepper oil is safe up to the maximum proposed use levels in complete feed of 5 mg/kg for chickens for fattening and other growing poultry, 8 mg/kg for laying hens and other laying/breeding birds kept for egg production/reproduction, 7 mg/kg for turkeys for fattening, 9.5 mg/kg for piglets and other growing *Suinae*, 11.5 mg/kg for pigs for fattening, 14 mg/kg for sows and dairy cows (and other dairy ruminants), 8.5 mg/kg in rabbits and 20 mg/kg in veal calves, cattle for fattening (and other growing ruminants), sheep, horses, salmonids (and other fin fish), dogs, cats and ornamental fish. For other growing species and non-food producing animals, the additive is considered safe at 5 mg/kg complete feed.

The supercritical extract of black pepper is safe up to the maximum proposed use levels in complete feed of 1.5 mg/kg for cats and dogs.

The black pepper oleoresin is safe up to the maximum proposed use levels in complete feed of 12.5 mg/kg for veal calves, 11.5 for cattle for fattening and other growing ruminants, sheep/goats and horses, 14 mg/kg for dogs 13.5 for mg/kg for salmonids and other fin fish and 51.5 for ornamental fish. For the other species, the calculated safe concentrations in complete feed are 1 mg/kg for chickens for fattening and other growing poultry, 1.4 mg/kg for laying hens and other laying/breeding birds kept for egg production/reproduction, 1.3 for turkeys for fattening, 1.7 mg/kg for piglets and other growing *Suinae*, 2 mg/kg for pigs for fattening, 2.5 mg/kg for sows, 2.4 mg/kg for dairy cows and other dairy ruminants, 1.5 mg/kg for rabbits, 3.8 mg/kg for cats. For all the other species, the additive is considered safe at 1 mg/kg complete feed. The FEEDAP Panel considers that the use of the additives in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

No concerns for consumers and for the environment were identified following the use of the additives (black pepper oil and black pepper oleoresin) at the use level considered safe in feed for the target animals.

Black pepper oil, supercritical extract of black pepper and black pepper oleoresin should be considered as irritant to skin and eyes, and as dermal and respiratory sensitisers.

Black pepper, its oil, oleoresin and preparations are recognised to flavour food. Since their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

5. **Recommendations**

The specification should ensure that the safrole concentration should be as low as possible and should not exceed 3 mg/kg of the essential oil and the supercritical extract and 10 mg/kg of the black pepper oleoresin.

6. **Documentation provided to EFSA/Chronology**

| Date       | Event                                                                 |
|------------|----------------------------------------------------------------------|
| 28/10/2010 | Dossier received by EFSA. Botanically defined flavourings from Botanical Group 06 - Laurales, Magnoliolae, Piperales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG) |
| 11/11/2010 | Reception mandate from the European Commission                         |
| 03/01/2011 | Application validated by EFSA – Start of the scientific assessment    |
| 01/04/2011 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: analytical methods* |
| 05/04/2011 | Comments received from Member States                                  |
| 20/04/2012 | Reception of supplementary information from the applicant             |
| 26/02/2013 | EFSA informed the applicant (EFSA ref. 7,150,727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission |
| 02/08/2013 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives |
Date | Event
--- | ---
24/06/2015 | Technical hearing during risk assessment with the applicant according to the ‘EFSA’s Catalogue of support initiatives during the life-cycle of applications for regulated products’: data requirement for the risk assessment of botanicals
18/12/2018 | EFSA informed the applicant that the evaluation process restarted
07/02/2019 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the consumer, safety for the user and environment*
21/05/2021 | Reception of supplementary information from the applicant (partial submission: pepper oil black)
05/10/2021 | Reception of supplementary information from the applicant (partial submission: supercritical extract)
06/01/2022 | Reception of supplementary information from the applicant (partial submission: pepper oleoresin)
16/02/2022 | The application was split and a new EFSA-Q-2022-00106 was assigned to the preparations included in the present assessment. Scientific assessment re-started
24/06/2022 | Reception of an amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to ylang oil, camphor white oil and cinnamon tincture
31/08/2022 | Reception of a second amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black
27/09/2022 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparation included in the present assessment. The assessment of other preparations is still ongoing

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**Abbreviations**

| ADFI | average daily feed intake |
| ADG  | average daily gain         |
| ADI  | average daily intake      |
| AFC  | EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food |
| ANS  | EFSA Scientific Panel on Additives and Nutrient Sources added to Food |
| BW   | body weight               |
| CAS  | Chemical Abstracts Service|
| CD   | Commission Decision       |
| CDG  | chemically defined group   |
| CEF  | EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CFU  | colony-forming unit       |
| CG   | chemical group             |
| CV   | coefficient of variation  |
| DM   | dry matter                 |
| ECHA | European Chemicals Agency  |
| EINECS | European Inventory of Existing Chemical Substances |
| EMA  | European Medicines Agency  |
| EUROL | European Union Reference Laboratory |
| FAO  | Food Agricultural Organisation |
| FCR  | feed conversion ratio      |
| FEEDAP | EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed |
| FFAC | Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures) |
| FGE  | food group evaluation     |
| FLAVIS | The EU Flavour Information System |
| FL-no | FLAVIS number              |
| GC-MS | gas chromatography–mass spectrometry |
| HACCP | hazard analysis and critical control points |
| IUPAC | International Union of Pure and Applied Chemistry |
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| LOD  | limit of detection        |
| Log Kow | logarithm of octanol–water partition coefficient |
| LOQ  | limit of quantification   |
| mbw  | metabolic body weight     |
| MCHC | mean corpuscular haemoglobin concentration |
| MCV  | mean corpuscular volume   |
| MIC  | minimum inhibitory concentration |
| MRL  | maximum residue limit     |
| MSDI | maximised survey-derived daily intake |
| MW   | molecular weight          |
| Acronym | Description                                      |
|---------|--------------------------------------------------|
| NOAEL   | no observed adverse effect level                 |
| NTP     | National Toxicology Program                      |
| OECD    | Organisation for Economic Co-operation and Development |
| RH      | relative humidity                                |
| SCAN    | Scientific Committee on Animal Nutrition         |
| SCF     | Scientific Committee on Food                     |
| TTC     | threshold of toxicological concern               |
| UF      | uncertainty factor                               |
| WHO     | World Health Organisation                        |
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for 18 compounds from botanically defined Group 06 (Laurales, Magnoniales, Piperales) – second amendment of the EURL report

In the period between the publication of the original EURL evaluation report [1] and the current date, eight flavouring compounds (cassia bark extract, cinnamon bark oleoresin, laurel leaves extract/oleoresin, boldo extract, boldo tincture, mace oil, nutmeg oleoresin and kawakawa tincture) were withdrawn from the grouped application FAD-2010-0218 Botanically defined flavourings from Group 06 - Laurales, Magnoliales, Piperales [2].

Upon request of DG SANTE, the EURL evaluated the new methods of analysis provided by the Applicant for three feed additives from the group, namely: ylang oil, camphor white oil and cinnamon tincture and recently issued a partial amendment of the original EURL report [3].

Following an additional request from EFSA [4], the EURL evaluated in the frame of this second amendment the new supplementary information provided by the Applicant related to the methods of analysis proposed for other six feed additives so-called: nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black which belong to the same grouped application.

Hereafter is the amended report on the evaluation of the new methods of analysis submitted by the Applicant and proposed for official control of the following feed additives: nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black. The updated recommendations of this amendment replace the ones stated for these six feed additives in the original report issued by the EURL [1].

For nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil and cassia oil, the Applicant proposed the quantification of their respective phytochemical markers, by gas chromatography coupled with flame ionisation detection (GC-FID), based on different available ISO standard methods.

Furthermore, the Applicant provided the analytical procedure with the specific operating conditions for the GC and applied it to the mentioned feed additives for the quantification of their respective phytochemical markers. According to the analytical procedure, 1 μL of the oil is injected into the GC using split ratio 100:1. The eluted compounds are detected by FID and the quantification is performed using the normalisation approach for the estimation of the area percentage of individual components (including also the phytochemical marker) in the obtained chromatograms.

(...)

Pepper oil black

According to the Applicant, pepper oil black is an essential oil obtained by super critical extraction (super critical extract) or by steam distillation (steam distilled) of the whole or broken unripe fruits of *Piper nigrum* L with a content of beta-caryophyllene (phytochemical marker) ranging from 8 to 30% (super critical extract) and from 12 to 40% (steam distilled) and expressed as the relative individual peak area in the chromatogram [11].

For the quantification of beta-caryophyllene in pepper oil black, the Applicant proposed a gas chromatography coupled with flame ionisation detection (GC-FID) method based on the standard ISO 3061:2004 for ‘Oil of black pepper (Piper nigrum L.)’ [12].

Furthermore, the description of the product and the range of beta-caryophyllene stated in the ISO 3061 standard corresponds to the range of the phytochemical marker as declared by the Applicant in the proposed specifications (steam distilled) [11].

In addition, the Applicant presented typical chromatograms of pepper oil black (super critical extract and steam distilled) demonstrating a good separation of the marker [12]. Moreover, the Applicant analysed the phytochemical marker (beta-caryophyllene) in five different batches of pepper oil black (super critical extract and steam distilled) leading to contents ranging from 11.5 to 15.6% for the super critical extract and from 25.4 to 28.2% for the steam distilled [11] being the latter within the range as specified in the ISO 3061 standard [12].

Given the performance characteristics and data currently available, the EURL recommends for official control the GC-FID method based on the ISO 3061 standard for the quantification of beta-caryophyllene (phytochemical marker) in pepper oil black.

(...
Pepper oleoresin black

According to the Applicant, *pepper oleoresin black* is an oleoresin obtained by solvent extraction of dried unripe fruits of *Piper nigrum* L’ with a content of *piperine* (phytochemical marker) ranging from 20 to 50% [19].

**Table A.1.** Performance characteristics of the GC-FID method for the quantification of the phytochemical marker (*piperine*) in the feed additive (*pepper oleoresin black*) [20]

|                  | **Batch 1** | **Batch 2** |
|------------------|-------------|-------------|
| content, % (relative area) | 38.4        | 38.6        |
| \(^a\text{RSD}_p\), % | 3           | 2.5         |
| \(^a\text{RSD}_{ip}\), % | 3.3         | 2.5         |

\(^a\text{RSD}_r\) and \(^a\text{RSD}_{ip}\): relative standard deviations for repeatability and intermediate precision, respectively.

\(^a\text{Recalculated by EURL [21].}\)

For the quantification of the *piperine* in *pepper oleoresin black* the Applicant proposed a high-performance liquid chromatography coupled to photometric detection (HPLC-UV) method based on the ISO 11027:1993 standard for ‘Pepper and pepper oleoresins – Determination of piperine content – Method using high performance liquid chromatography’ [20].

According to the procedure provided by the Applicant, the *pepper oleoresin black* (0.2 g) is mixed with methanol and stirred for 5 min. After centrifugation, an aliquot of the supernatant is further diluted with methanol and injected directly into the HPLC system. The phytochemical marker (*piperine*) is then separated by reversed-phase HPLC using a gradient elution. *Piperine* is quantified at 280 nm using an external standard curve [20].

The Applicant verified the method proposed for the analysis of the phytochemical marker (*piperine*) following the ‘EURL–FA Validation and verification technical guide for Sensory feed Additives – flavouring compounds 2(b) from botanical origin’ [10]. Table A.1 shows a summary of the relevant performance characteristics obtained from the verification study. The precision values, recalculated by the EURL [24] from the verification study, ranged from 2.5 to 3.3% for the determination of *piperine* in *pepper oleoresin black* [20].

Based on the experimental evidences provided the EURL recommends for official control the HPLC-UV method based on the generic ISO 11027 standard for the quantification of *piperine* (phytochemical marker) in *pepper oleoresin black*. 