SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 7 May 2020

doi: 10.2903/j.efsa.2020.6147

Safety and efficacy of essential oil, oleoresin and tincture from *Zingiber officinale* Roscoe when used as sensory additives in feed for all animal species

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Abstract

Following a request from the European Commission, the EFSAPanel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of essential oil, oleoresin and tincture from *Zingiber officinale* Roscoe when used as sensory additives in feed for all animal species. The FEEDAPPanel concludes that the additives under consideration are safe for the target species at the following use levels: (i) ginger essential oil up to the maximum proposed use level of 80 mg/kg for veal calves (milk replacer) and 20 mg/kg complete feed (or 20 mg/L water for drinking) for all other species; (ii) ginger oleoresin at the maximum proposed concentrations of 20 mg/kg complete feed for fish, sheep, goats and horses and of 1 mg/kg for pets. For the remaining species, the calculated maximum safe concentration of ginger oleoresin in feed is less than that proposed use level and ranges from 5 mg/kg complete feed for chickens for fattening to 21 mg/kg for veal calves; (iii) ginger tincture at the maximum proposed concentrations of 1.6 mL/kg complete feed for horses and 0.26 mL/kg for dogs. For poultry species, the calculated maximum safe dose ranges between 0.2 and 0.3 mg/L water for drinking. No concerns for consumers were identified following the use of the additives up to the highest safe level in animal nutrition. The additives should be considered as irritants to skin and eyes and the respiratory tract and as a skin sensitisers. The use of the additives in feed is not expected to pose a risk for the environment. Since ginger and its preparations are recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

Keywords: sensory additives, flavouring compounds, ginger, *Zingiber officinale* Roscoe, safety

Requestor: European Commission

Question number: EFSA-Q-2017-00533

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Acknowledgements: The EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) wishes to thank the following for the support provided to this scientific output: Montserrat Anguita, Jaume Galobart and Fabiola Pizzo.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Westendorf J, Gregoretti L, Manini P and Dusemund B, 2020. Scientific Opinion on the safety and efficacy of essential oil, oleoresin and tincture from *Zingiber officinale* Roscoe when used as sensory additives in feed for all animal species. EFSA Journal 2020;18(6):6147, 31 pp. https://doi.org/10.2903/j.efsa.2020.6147

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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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 and 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 7 years after the entry into force of this Regulation.

The European Commission received a request from the Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of nine preparations (namely turmeric oil, oleoresin, extract (sb) and tincture from *Curcuma longa* L., cardamom oil from *Elettaria cardamomum* (L.) Maton, ginger oil, oleoresin, tincture and extract from *Zingiber officinale* Roscoe) belonging to botanically defined group (BDG) 9 - *Zingiberales*, when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the course of the assessment, this application was split and the present opinion covers only four out of the nine preparations under application: ginger oil, ginger oleoresin, ginger tincture and ginger extract from *Z. officinale* for all animal species. During the assessment, the applicant withdrew the application for ginger extract.[3] This preparation is excluded from the present assessment.

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) and under Article 10(2) (re-evaluation of an authorised 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.[4]

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 ginger oil, ginger oleoresin and ginger tincture from *Z. officinale*, 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 five preparations belonging to botanically defined group (BDG) 9 - *Zingiberales* under application are assessed in separate opinions.

1.2. Additional information

The three preparations under assessment, namely ginger oil, ginger oleoresin and ginger tincture from *Z. officinale*, are currently authorised as feed additives and listed 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.

There is no specific EU authorisation for any *Z. officinale* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008[5], flavourings preparations produced from food or food ingredients with flavouring properties may be used without an evaluation and approval as long as 'they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer'.

[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, p. 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, 1050 Brussels, Belgium.

[3] On 27 February 2019, EFSA was informed about the withdrawal of the application on ginger extract.

[4] On 26 February 2013, EFSA duly informed the applicant (EFSA ref. 7150727) 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. On 24 July 2017, EFSA informed the applicant that the evaluation process restarted.

[5] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.

The European Medicines Agency (EMA, 2012) assessed *Z. officinale*, rhizome, as herbal medicinal product in the form of powdered herbal preparation. The European Food Safety Authority (EFSA NDA Panel, 2010) assessed the validity of the health claim on maintaining mobility of joints.

'Ginger' (Zingiberis rhizoma) is described in a monograph of the European Pharmacopoeia 10 (European Pharmacopoeia, 2019). It is defined as the dried, whole or cut rhizome of *Z. officinale*, with the cork removed, either completely or from the wide flat surfaces only, with a minimum content of essential oil of 15 mL/kg anhydrous drug.

The preparations from *Z. officinale* are listed in the book of botanical flavourings of the Council of Europe with the number CoE 489/ Class N2- roots.[6]

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[7] in support of the authorisation request for the use of ginger oil, ginger oleoresin and ginger tincture from *Z. officinale* as feed additives.

The 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 use the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations from *Z. officinale*.[8]

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 the nine feed additives from botanically defined flavourings group 09 (BDG 09) – Zingiberales.[9] The EURL delivered in 2018 an evaluation report related to the Botanically Defined Flavourings Group BDG 09 – Zingiberales. In this report, only analytical methods for cardamom oil were evaluated. On 25 February 2020, the EURL delivered an addendum to the above-mentioned report, in which the remaining feed additives included in this group were evaluated. In particular, regarding the feed additives subject of the present scientific opinion, the method of analysis for alpha-zingiberene, beta-sesquiphellandrene and ar-curcumene in ginger oil, and the methods of analysis for total gingerols and total shogaols in ginger oleoresin and ginger tincture were evaluated. For the ninth feed additive of this grouped application, namely ginger extract, the European Commission had accepted the request from the Applicant to withdraw the application for this feed additive. Therefore, the analytical methods for ginger extract were not evaluated in this addendum. The full report including the addendum is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2010-0335.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of ginger oil, ginger oleoresin and ginger tincture from *Z. officinale* is in line with the principles laid down in Regulation (EC) No 429/2008[10] 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, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of

[6] Section II, Table II. 1, p6
[7] FEED dossier reference: FAD-2010-0420.
[8] Technical dossier/Supplementary information February 2018/ 2018-01-30_SInReply_cardamom.
[9] Reference: EURL evaluation report related to FAD-2010-0335 – Botanically Defined Flavourings Group BDG 09 - Zingiberales (JRC F.5/CvH/ZE/AS/Ares (2018)5225574) issued on 11/10/2018.
[10] 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.

use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017b), 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).

3. Assessment

The additives under assessment are ginger oil, ginger oleoresin and ginger tincture from *Zingiber officinale* Roscoe and are intended for use as sensory additives (flavourings) in feed and water for drinking for all animal species.

3.1. Origin and extraction

Ginger (*Zingiber officinale* Roscoe) is a perennial flowering plant which belongs to the Zingiberaceae family.

The plant is probably native to Southeast Asia, but the wild type is no longer found and present-day ginger exists only as a cultigen. It is widely distributed and cultivated throughout Southeast Asia, and the Indian subcontinent and also commercially grown in Africa, China, Jamaica and other tropical parts of the world. The parts of the plant used for production of various culinary and medicinal preparations from the species are the fresh and/or dried rhizomes, which are designated by the name 'ginger' as the plant itself.[11]

The plant components present in the different preparations depend on the selectivity of the extraction process. The different extraction processes used for the additives which are the subject of this opinion, namely ginger essential oil, oleoresin and tincture, are described under their respective headings.

3.2. Ginger essential oil

This application concerns the essential oil derived by steam distillation from the dried rhizomes of *Z. officinale* of Indian and Chinese origin. Briefly, steam is passed through the dried minced material from an external source or generated by boiling water below the material or by boiling water in which the material is immersed. The steam carries up the volatile constituents which are condensed. The essential oil is then separated from water by decantation.

The amount of the essential oil present in rhizomes ranges between 1 and 5%, mainly containing α-zingiberene (70% in fresh and 20–30% in dried rhizomes).[11] The molecular structures of the main components of the essential oil are shown in Figure 1.

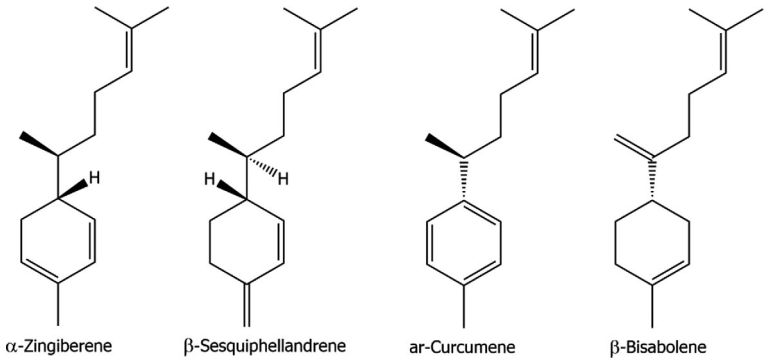

Figure 1: Molecular formula of the main components of ginger essential oil

3.2.1. Characterisation of ginger essential oil

The essential oil under assessment is a pale yellow to amber clear mobile liquid with a characteristic aroma of ginger. In 11 batches of the additive (five originating from China and six from India), the specific optical rotation at 25°C ranged between −38° and −32° (specification: −50° to −26°), the

[11] Technical dossier/Section II/Annex_II_4.

refractive index between 1.488 and 1.490 (specification: 1.484–1.498) and the density (25°C) between 874 and 881 kg/m^3 (specification: 879–890).[12] Ginger oil is identified with the single Chemical Abstracts Service (CAS) number 8007-08-7, European Inventory of Existing Chemical Substances (EINECS) number 283-634-2 and Flavour Extract Manufacturers Association (FEMA) 2522.

The product specifications are based on the International Standard developed by the International Organisation for Standardization (ISO) 16928:2014 for essential oil of ginger [*Zingiber officinale*], which were adapted to reflect the concentrations of the main components of the essential oil, namely α-zingiberene (29–40%), β-sesquiphellandrene (8–14%), ar-curcumene (5–12%), α-farnesene (4–10%), camphene (2–10%) and β-bisabolene (2–9%). Analysis of 11 batches of the additive[13] showed compliance with these specifications (Table 1). These six compounds account for about 70.8% on average (range 69.6–71.8%) of the product, expressed as area the area per cent (%) of the gas chromatographic (GC) profile.

Table 1: Major constituents of the essential oil from the dried rhizomes of *Zingiber officinale* Roscoe as defined by the ISO standard: specifications and batch to batch variation based on the analysis of 11 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				Percentage of oil	
EU register name	**CAS No**	**FLAVIS No**	**Specification GC Area %**	**Mean[(a)]**	**Range**
α-Zingiberene	495-60-3	–	29–40	36.78	33.82–39.20
β-Sesquiphellandrene	20307-83-9	–	8–14	10.25	9.22–11.23
ar-Curcumene	644-30-4	–	5–12	9.51	8.52–11.01
α-Farnesene	18794-84-8	01.014	4–10	6.84	4.45–9.32
Camphene	79-92-5	01.009	2–10	3.80	2.97–6.38
β-Bisabolene	495-61-4	01.028	2–9	3.65	2.47–4.24
Total				70.84	69.58–71.79

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Mean calculated on 11 batches.

The applicant provided the full characterisation of the 11 batches obtained by gas chromatography coupled with a flame ionisation detector (GC-FID) and mass spectrometry detector (GC-MS).[13] In total, 103 constituents were detected, 91 of which were identified and accounted on average for 94.8% (92.8–95.4) of the product (as the GC area). Besides the six compounds indicated in the product specifications (accounting for 70.8% of the product), 13 other compounds were detected at levels > 0.5% and are listed in Table 2. These 19 compounds > 0.5% together account on average for 84.9% (83.2–86.0%) of the product. The remaining 72 compounds (ranging between 0.001% and 0.5% and accounting together for about 15% of the product) are listed in the footnote.[14]

[12] Technical dossier/Supplementary information August 2018/Annex_II_ SIn_Reply_Ginger_Oil_CoA.
[13] Technical dossier/Supplementary information August 2018/Annex_I_SIn _Reply_Ginger_Oil_Batch_data.
[14] Additional constituents: compounds (19) ranging between 0.2% and < 0.5%: γ-Muurulene, 6-methyl-5-hepten-3-one, myrcene, decanal, α-copaene, 2-undecanone, 10-epi-γ-eudesmol, geraniol, germacrene B, γ-elemene, β-eudesmol, cyclosativene, rosefuran epoxide, sesquisabinene, α-funebrene, neral, calamenene, linalool and hexanal; compounds (12) ranging between 0.1% and < 0.2%: β-Curcumene, α-phellandrene, β-pinene, α-cadinol, tricyclene, α-eudesmol, bornyl acetate, rosefuran, β-bisabolol, terpinolene, δ-elemene and octanal; compounds (15) ranging between 0.05% and 0.1%: β-Cubebene, epi-cubebol, *p*-cymene, camphor, cadina-1,4-diene (trans), citronellal, α-bergamotene (trans), heptan-2-one, geranyl acetone, methyl heptyl ketone, α-cubebene, α-bisabolol, sabinene, p-2-menthen-1-ol cis and 2-nonanol; compounds (26) < 0.05%: Toluene, α-fenchene, δ-carene, p-mentha-1(7),8-diene, 2,3-dehydro-1,8-cineole, γ-terpinene, β-ocimene (trans), 2-heptyl acetate, 2-heptanol, melonal (2,6-dimethyl-5-en-2-one), nonanal, perillene, 1-isoprenyl-4-methylbenzene, linalool oxide cis, 6-methylhept-5-en-2-ol, non-3-en-2-one, 7-epi-sesquithujene, α-santalene, 2-tridecanone, myrtenal, p-2-menthen-1-ol (trans), β-calacorene, caryophyllene oxide, palmitic acid, 2-pentyl furan and β-ocimene cis.

Table 2: Constituents of the essential oil from the dried rhizomes of *Zingiber officinale* Roscoe accounting for > 0.5% of the composition (based on the analysis of 11 batches) 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			Percentage of oil	
EU register name	**CAS No**	**FLAVIS No**	**Mean[(a)]**	**Range**
β-Phellandrene	555-10-2	01.055	3.04	2.47–4.22
Pin-2(3)-ene (α-Pinene)	80-56-8	01.004	1.44	1.17–2.38
1,8-Cineole	470-82-6	03.001	1.33	1.16–1.58
δ-Cadinene	29350-73-0	01.021	1.31	0.71–1.83
Germacra-1(10),4(14),5-triene	23986-74-5	01.042	1.17	0.91–1.46
D,L-Borneol	507-70-0	02.016	1.05	0.88–1.15
β-Elemene	33880-83-0	–	1.00	0.81–1.08
d-Limonene	5989-27-5	01.045	0.80	0.65–1.04
α-Terpineol	98-55-5	02.014	0.78	0.56–1.06
(-)-α-Elemol	639-99-6	02.149	0.57	0.49–0.65
trans-Nerolidol	7212-44-4	02.018	0.56	0.47–0.71
Farnesene (α and β)	18794-84-8	01.041	0.53	0.24–0.93
Alloaromadendrene	25246-27-9	–	0.52	0.31–0.66
Total			14.09	13.52–15.94

EU: European Union; CAS no.: Chemical Abstracts Service number; Flavis number: EU Flavour Information System numbers.
(a): Mean calculated on 11 batches.

Twenty-two peaks were detected but not identified, accounting in total for 3.60–6.34% (4.39% on average), with the single highest peak accounting on average for < 0.43% of the oil.

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *Z. officinale* and its preparations.[15] Perillene, 3-(4-methyl-3-pentenyl)-furan, CAS number 539-52-6, is a furan derivative that carries an alkenyl side chain in the 3-position. The occurrence of perillene has been reported in ginger rhizome essential oil. Analysis of the 11 batches showed an average content of 0.02% of perillene.

3.2.1.1. Impurities

No information on the concentrations of undesirable compounds in the essential oil was given. The applicant makes reference to the 'periodic testing' of some representative flavourings premixtures for heavy metals (mercury, cadmium and lead), arsenic, fluoride, dioxins and dioxin-like polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorous pesticides, aflatoxin B1, B2, G1, G2 and ochratoxin A. Since ginger essential oil is produced by steam distillation, the likelihood of any measurable carry-over of heavy metals is low except for mercury (Tascone et al., 2014).

3.2.1.2. Shelf-life

The typical shelf-life of ginger essential oil is stated to be at least 18 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light).[16] No stability studies were performed for the essential oil.

3.2.1.3. Conditions of use

Ginger essential oil is intended to be added to feed and water for drinking for all animal species without withdrawal period.[17] The maximum proposed use level is 20 mg/kg complete feed for all species, except veal calves (milk replacer) and dairy cows, for which the maximum proposed use level is up to 150 mg/head and day which corresponds to 80 and 7.5 mg/kg (on dry matter basis), respectively. No specific use level has been proposed by the applicant for the use in water for drinking.

[15] Technical dossier/Supplementary information August 2018/Literature search_Zingiber officinale Rosc-final.
[16] Technical dossier/Supplementary information August 2018/Annex IV_Turm_Oil_Specifications.
[17] During the assessment, the applicant has clarified that insoluble additives are properly formulated to allow homogeneous distribution in water for drinking.

3.2.2. Safety

The assessment of safety is based on the maximum use level proposed by the applicant.

3.2.2.1. Absorption, distribution, metabolism and excretion

As no specific studies on absorption, distribution, metabolism and excretion (ADME) with the oil under assessment were provided, the ADME of the individual constituents is therefore considered.

With the exception of camphene, which is a bicyclic monoterpenoid, the major components of the essential oil (α-zingiberene, ar-curcumene, β-sesquiphellandrene, β-bisabolene and α-farnesene) are structurally related sesquiterpenes (Figure 1). Other minor constituents are also aliphatic mono- or sesquiterpenes. Mono and sesquiterpenes are lipophilic compounds, which are expected to be rapidly absorbed from the gastro-intestinal tract and oxidised to polar oxygenated metabolites (by the cytochrome P450 enzymes, alcohol dehydrogenases and aldehyde dehydrogenases). The resulting hydroxylated metabolites may be excreted as glucuronide- and sulfate-conjugates or undergo further oxidation, yielding more polar metabolites that are also excreted in conjugated form in the urine and bile. Oxidation of the double bonds leads to epoxide intermediates which are rapidly detoxified either by hydrolysis to yield diols or by conjugation with glutathione. The enzymes involved in the biotransformation pathways of these compounds are present in all the target (food-producing and non-food producing) species (reviewed in EFSA FEEDAP Panel, 2016a).

3.2.2.2. Toxicological studies

The applicant submitted a subchronic 90-day oral toxicity rat study performed with an essential oil from rhizome of *Zingiber officinale* (Jeena et al., 2011). Analysis shows that the essential oil tested is similar in composition and content to the essential oil under assessment (Table 3). Among the major components, the main differences are due to the different percentage of β-bisabolene, α-zingiberene and α-farnesene. However, these three compounds, accounting together for about 45% of the composition of the oils, are structurally related (see Figure 1) and have a similar toxicological profile.

Table 3: Comparison of the test item used in the subchronic oral toxicity study (A) and the ginger essential oil under assessment (B)

Constituent	Essential oil A (%)	Essential oil B (%)
α-Zingiberene	31.08	36.8 (33.8–39.2)
β-Sesquiphellandrene	14.02	10.2 (9.2–11.2)
ar-Curcumene	15.35	9.5 (8.5–11.0)
α-Farnesene	–	6.8 (4.4–9.3)
Camphene	5.14	3.8 (3.0–6.4)
β-Bisabolene	13.81	3.7 (2.5–4.2)
β-Phellandrene	–	3.0 (2.5–4.2)
Germacra-1(10),4(14),5-triene	2.40	1.2 (0.9–1.5)
D,L-Borneol	1.14	1.0 (0.9–1.1)
β-Elemene	2.14	1.0 (0.8–1.1)
Alloaromadendrene	0.34	0.5 (0.3–07)
	85.08	77.5

A total of 50 male and female Wistar rats (5 males and 5 females per group) were given 0 (control), 0 (vehicle control), 100, 250 or 500 mg essential oil A/kg body weight (bw) per day by oral gavage for 90 days. There were no deaths and no significant differences in growth between groups. The results of haematology, blood chemistry, gross pathology and histology showed no evidence of any treatment-related adverse effects. From this study, the highest dose tested (500 mg/kg bw per day) was identified by the authors as the no observed adverse effect level (NOAEL). The FEEDAP Panel agrees with the conclusions of the authors of the study.

Ginger essential oil (A) was also tested for the induction of reverse mutations in Salmonella Typhimurium tester strains TA1535, TA98, TA100 and TA102 with or without metabolic activation, at five concentration levels up to 3 mg/plate. No indication of mutagenic activity was observed in any of the experimental conditions (Jeena et al., 2014).

The FEEDAP Panel notes that 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). The ginger essential oil under assessment is well characterised (up to 94.8%) and all the components > 0.5% (except β-elemene and alloaromadendrene, for which no alerts were identified by QSAR analysis) have been assessed by EFSA for use in feed and/or food, and are currently authorised for food[18] and feed[19] uses. In these assessments, it was established that these compounds were not genotoxic. The remaining components were screened with OECD QSAR Toolbox and no alert were identified for *in vitro* mutagenicity (Ames test), for genotoxic and non-genotoxic carcinogenicity and for other endpoints. The Panel notes that at the maximum proposed use levels in feed, none of the components of the essential oil ranging in concentrations between 0.001% and 0.5%, including perillene, is considered of concern, as they are below the threshold of the corresponding Cramer Class, according to the threshold of toxicological concern (TTC) approach.

3.2.2.3. 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 these data, the approach to the safety assessment of the whole mixture can be based on read-across from a sufficiently similar mixture (EFSA SC, 2019a). The FEEDAP Panel considers the composition of the ginger essential oil tested in the 90-day study (Jeena et al., 2011) sufficiently similar to that of the oil under assessment. Therefore, the FEEDAP Panel identified the NOAEL of 500 mg/kg bw per day from this 90-day study as a suitable reference point to assess the safety of the ginger essential oil under assessment.

Applying an uncertainty factor (UF) of 100 to the NOAEL, the safe daily dose for the target species was derived following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), and thus, the maximum safe feed concentration was calculated (Table 4).

Since glucuronidation of the hydroxylated or oxygenated metabolites of the individual constituents of ginger essential oil is an important metabolic pathway facilitating the excretion of these compounds (see Section 3.2.2.1), the calculation of safe concentrations in cat feed needs an additional UF of 5. This factor is due to the unusually low capacity for glucuronidation in cats (Court and Greenblatt, 1997).

Table 4: Maximum safe concentration in feed for different target animals for ginger essential oil

	Body weight (kg)	Feed intake (g DM/day)	Daily feed intake (g DM/kg bw)	Maximum safe concentration (mg/kg feed)(1)
Chickens for fattening	2	158	79	56
Laying hens	2	106	53	83
Turkeys for fattening	3	176	59	75
Piglets	20	880	44	100
Pigs for fattening	60	2,200	37	120
Sow lactating	175	5,280	30	146
Veal calves (milk replacer)	100	1,890	19	233
Cattle for fattening	400	8,000	20	220
Dairy cows	650	20,000	31	143
Sheep/goat	60	1,200	20	220
Horse	400	8,000	20	220
Rabbit	2	100	50	88
Salmon	0.12	2.1	18	251
Dogs	15	250	17	264
Cats(2)	3	60	20	55
Ornamental fish	0.012	0.054	5	978

(1): Complete feed containing 88% DM, milk replacer 94.5% DM.
(2): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

[18] 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, p. 1.

[19] 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

The FEEDAP Panel concludes that ginger essential oil added to the feed of all animal species is safe at the maximum proposed use level of 20 mg/kg complete feed with a margin of safety of at least 2.7. The higher maximum use level of 80 mg/kg for veal calves (milk replacer) is also considered safe for this species category.

No specific proposals have been made by the applicant for the use level in water for drinking. Therefore, the FEEDAP Panel considered the same use level in water for drinking (20 mg/L) as proposed for feed (20 mg/kg). When used at 20 mg/L water for drinking, the intake of the additive via water would be two to three times higher than the intake via feed for poultry, pigs and rabbits (EFSA FEEDAP Panel, 2010). Considering the magnitude of the margin of safety, a concentration of 20 mg essential oil/L water for drinking is considered safe for all animal species.

3.2.2.4. Safety for the consumer

Rhizomes of *Z. officinale* and their preparations including the essential oil are added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2010) cites values of 3 mg/kg bw per day for ginger rhizome and 0.0263 mg/kg bw per day for ginger essential oil.

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 ginger essential oil are expected to be extensively metabolised and excreted in the target species (see Section 3.2.2.1). 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 ginger rhizome and its preparations in food (Burdock, 2010), it is unlikely that consumption of products from animals given ginger essential oil at the proposed maximum use level would significantly increase human background exposure.

Consequently, no safety concern would be expected for the consumer from the use of ginger essential oil up to the highest safe use level in feed.

3.2.2.5. Safety for the user

No specific data were provided by the applicant regarding the safety of the additive for users.

Ginger oil has been notified to the European Chemical Agency (ECHA) for classification and labelling according to Classification Labelling and Packaging (CLP)[20] as aspiratory toxic (H304), skin irritant (H315), skin sensitiser (H317), eye irritant (H319) and respiratory irritant (H335).

3.2.2.6. Safety for the environment

The addition of naturally occurring substances that will not result in a substantial increase of the concentration in the environment is exempt from further assessment (EFSA, 2008). This exemption applies to botanical preparations from plants native to Europe. However, *Z. officinale* is not native to Europe. Therefore, the safety for the environment is assessed based on the individual components of the essential oil.

Sixteen identified constituents of ginger essential oil (camphene, β-phellandrene, pin-2(10)-ene, 1,8-cineole, borneol, d-limonene, β-myrcene, α-terpineol, nerolidol, decanal, 6-methyl-5-hepten-3-one, 2-undecanone, geraniol, neral, linalool and hexanal) 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.

Most of the remaining major and minor constituents present in ginger essential oil have not been evaluated by EFSA with respect to its safety for the environment. The major and minor constituents are generally aliphatic mono or sesquiterpenes, partially with functional groups. The hydrocarbon derivatives are chemically related to the substances evaluated by EFSA in CG 31 for use in animal feed (EFSA FEEDAP Panel, 2015, 2016a) for which EFSA concluded that they were 'extensively metabolised by the target species and excreted as innocuous metabolites or carbon dioxide. Average feed levels of constituents of ginger essential oil in animal feed are much lower than the authorised use levels for CG 31 substances. Therefore, no risk for the safety of the environment is foreseen'. The same conclusion applies to the substances chemically related to those evaluated in CG 31.

[20] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353 of 31.12.2008, p.1

The use of ginger essential oil up to the highest safe level in feed is not expected to pose a risk for the environment.

3.3. Ginger oleoresin

Ginger oleoresin is obtained by solvent extraction of dried rhizomes of *Z. officinale*, [redacted]

Besides lipids and proteins, the main components of ginger oleoresin are gingerols (6-, 8- and 10-gingerol), shogaols (6-, 8- and 10-shogaol) and volatile components from the essential oil. The molecular structures of gingerols and shogaols are shown in Figure 2. The shogaols are formed by dehydration of gingerols and their content in the mixture depends on the conditions of the processing of the raw material.

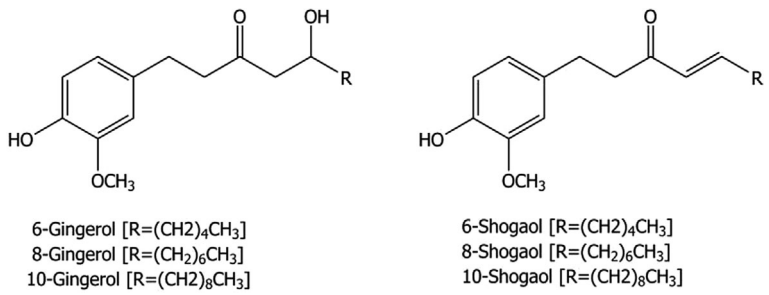

Figure 2: Molecular formula of the main components of ginger oleoresin

3.3.1. Characterisation

The additive is a dark brown viscous liquid with characteristic odour and pungent flavour of ginger. It contains by specification 25–35 mL (22.0–30.8 g) essential oil/100 g, 0.5–8% total gingerols and 3–6% shogaols.[22]

Table 5 summarises the results of proximate analysis[23] and Table 6 the characterisation of the organic fraction in six batches of the additive from three different producers (from Indian origin). The content of essential oil was also determined by distillation, giving consistent results with those obtained in the proximate analysis.[24] Individual gingerols and shogaols were determined by high-performance liquid chromatography (HPLC) with UV detector,[25] the essential oil fraction was characterised by GC-MS.[26] The relative concentration of the components of the essential oil (as g/100 mL) was converted in g/100 g considering the average density of the essential oil (880 kg/m^3).

[redacted]

[22] Technical dossier/Supplementary information March 2019.
[23] Technical dossier/Supplementary information March 2019/Annex_V_Ging_Oleo_Proximate.
[24] Technical dossier/Supplementary information March 2019/Annex_VI_Ging_Oleo_Essential oil.
[25] Technical dossier/Supplementary information March 2019/Annex_IV_Ging_Oleo_Gingerol_Shogaol.
[26] Technical dossier/Supplementary information March 2019/Annex_VII_Ging_Oleo_GC-MS.

Table 5: Proximate analysis of ginger oleoresin (*Zingiber officinale* Roscoe) based on the analysis of six batches (mean and range). The results are expressed as % (w/w)

Constituent	Mean[(a)]	Range
	% (w/w)	% (w/w)
Humidity, volatiles	27.6	25.7–28.6
Ash	0.12	< 0.1–0.13
Lipids	32.6	15.7–43.0
Protein	5.28	4.29–8.46
Fibre	0.92	0.5–1.9
Total	66.5	61.1–77.8

(a): Mean calculated on six batches.

Table 6: Characterisation of the fraction of secondary metabolites of ginger oleoresin (*Zingiber officinale* Roscoe) based on the analysis of six batches (mean and range). The results are expressed as % (w/w) of ginger oleoresin

Constituent	CAS No	FLAVIS No	Mean[(a)]	Range
			% (w/w)	% (w/w)
Gingerols (total, HPLC)			3.25	0.94–7.56
6-Gingerol	23513-14-6	–	1.68	0.30–4.21
8-Gingerol	23513-08-8	–	0.62	0.24–1.25
10-Gingerol	23513-15-7	–	0.95	0.40–2.10
Shogaols (total, HPLC)			4.64	3.94–5.57
6-Shogaol	555-66-8	–	3.08	2.49–3.98
8-Shogaol	104186-07-4	–	0.62	0.47–0.89
10-Shogaol	36752-54-2	–	1.51	0.91–3.94
Total			7.88	4.95–13.1
Essential oil (total, distillation)			26.83	25–30
Zingiberene	495-60-3	–	5.27	2.30–7.23
Camphene	79-92-5	01.009	2.49	0.01–4.51
β-Sesquiphellandrene	20307-83-9	–	2.29	0.87–3.45
ar-Curcumene	644-30-4	–	1.48	0.71–2.01
β-Phellandrene	555-10-2	01.055	1.45	0.29–2.13
d-Limonene	5989-27-5	01.045	1.41	0.03–3.93
β-Bisabolene	495-61-4	01.028	1.28	0.52–1.83
Pin-2(3)-ene	80-56-8	01.004	1.21	0–2.08
α-Farnesene	502-61-4	01.040	1.17	0.43–1.70
γ-Cadinene	39029-41-9	–	0.62	0.29–1.0
Germacrene D	23986-74-5	01.042	0.61	0–1.02
Pin-2(10)-ene	127-91-3	01.003	0.51	0–1.53
Vanillyl acetone (zingerone)	122-48-5	07.005	0.33	0.18–0.46
Isoborneol	124-76-5	02.059	0.31	0.18–0.46
Hexadecanoic acid	57-10-3	08.014	0.24	0.19–0.33
6,10-Dodecadien-1-yn-3-ol, 3,7,11,trimethyl	2387-68-0	–	0.23	0.14–0.40
Sum identified components			20.9	18.5–24.4
Other identified	–	–	4.83	3.82–5.98
Total unidentified			0.65	0.11–3.12
Total identified (gingerols and shogaols + volatiles)			34.72	30.9–43.1

CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System number.
(a): Mean calculated on six batches.

Overall, it is estimated that the results of proximate analysis (including volatiles) together with gingerols and shogaols account on average for 74.4% (58.1–90.0%) of the composition of oleoresin.

3.3.1.1. Impurities

Data on chemical and microbial impurities were provided in at least two batches of ginger oleoresin.[27] The concentrations of heavy metals were below the corresponding limit of quantification (LOQ), with the exception of mercury (< 0.002–0.058 mg/kg) in three batches. Mycotoxins (aflatoxins B1, B2, G1 and G2) were below the LOQ and pesticides were not detected in a multiresidue analysis in four batches.

In two batches, the sum of polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofuran (PCDF) and dioxin-like polychlorinated biphenyls (PCBs) ranged between 2.17 and 3.28 pg/g. None of the data on chemical impurities raised concerns.

Analysis of microbial contamination of three batches of ginger oleoresin indicated that *Salmonella* spp. was absent in 25 g, *Enterobacteriaceae* were < 10^1 colony-forming unit (CFU)/g, total viable count, yeasts, moulds were < 10^2 CFU/g.

3.3.1.2. Shelf-life

The typical shelf-life of pure flavouring compounds is stated to be at least 12 months, when stored in tightly closed containers under standard conditions. No stability studies were performed for the oleoresin.

3.3.1.3. Conditions of use

Ginger oleoresin is intended to be added to feed and water for drinking for all animal species without withdrawal period.[17] The applicant proposed a minimum use level of 0.2–0.4 mg/kg complete feed for poultry and 1 mg/kg for the other species. The maximum proposed use level is 20 mg/kg complete feed for poultry, pigs, horses and fish, 150 mg/ head and day for veal calves (milk replacer), cattle for fattening and dairy cows and 1 mg/kg complete feed for pets. No use level has been proposed by the applicant for the use in water for drinking.

3.3.2. Safety

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

3.3.2.1. Absorption, distribution, metabolism and excretion

The ADME information of the volatile fraction of the ginger oleoresin is described in Section 3.2.2.1.

Information for the most prominent compounds of the non-volatile fraction (i.e. gingerols and shogaols) taken from the literature was provided by the applicant.

Rats were given 50 mg 6-gingerol/kg bw by direct stomach intubation and bile and urine were collected. In one group of rats, cannulation of the bile duct was performed and bile was collected. The authors identified three main metabolic pathways of 6-gingerol: glucuronidation, ω-oxidation and β-oxidation of the phenolic side chain. Sixty hours after administration, 6-gingerol was excreted as glucuronide conjugate in the bile at about 48% of the administered dose. The glucuronide was not detected in urine, but six other minor metabolites (vanillic acid, ferulic acid, (S)-(+)-4-hydroxy-6-oxo-8-(4-hydroxy-3-methoxyphenyl) octanoic acid, 4-(4-hydroxy-3-methoxy-phenyl)butanoic acid, 9-hydroxy [6]-gingerol and (S)-(+)-[6]-gingerol) were detected in urine and accounted for 16% of the administered dose (Nakazawa and Oshawa, 2002). In another group of rats, gut sterilisation caused a reduction of the six minor metabolites excreted in the urine. Incubation of 6-gingerol with rat liver *in vitro* resulted in the formation of 6-gingerol-glucuronide, 9-hydroxy-6-gingerol and gingerdiol. These experiments proved that intestinal microbiota and liver both contribute to the metabolism of 6-gingerol and its subsequent excretion.

The metabolic fate of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol in humans was investigated in a clinical trial (Zick et al., 2008). Volunteers received encapsulated dry extract of ginger rhizome. A capsule of 250 mg extract contained 5.38 mg 6-gingerol, 1.80 mg 8-gingerol, 4.19 mg 10-gingerol and 0.92 mg 6-shogaol. Single oral doses were administered to different groups ranging from 100 mg to 2.0 g ginger rhizome extract. Blood samples were obtained at 15 min and further nine time points up

[27] Technical dossier/Supplementary information March 2019/Annexes X, XI and XII. LOQ for heavy metals and arsenic: < 0.01 mg/kg for lead and arsenic, < 0.002 mg/kg for mercury and cadmium; LOQ for individual pesticides: 0.1 mg/kg; LOQ for mycotoxins: < 0.1 μg/kg for aflatoxins B1, B2, G1 and G2.

to 72 h. Plasma levels of free gingerols/shogaol and their conjugates were determined by HPLC (limit of quantification of the method ranged from 0.1 μg/mL to 0.25 μg/mL). No participant had detectable free 6-gingerol, 8-gingerol, 10-gingerol or 6-shogaol in plasma at any time point. After enzymatic hydrolysis of plasma with glucuronidase/sulfatase, 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol were detected in samples collected up to 4 h and 6-gingerol at 8 h in a volunteer given a 2 g dose. The 6-gingerol sulfate conjugate was detected above the 1.0-g dose, but there were no detectable 10-gingerol or 6-shogaol sulfates except for one participant with detectable amounts of 8-gingerol sulfate. The maximum plasma concentrations, calculated as the free form of the compounds after administration of 1 g, 1.5 g and 2 g of the preparation ranged from 0.4 to 1.69 μg/mL for 6-gingerol, from 0.1 to 0.23 μg/mL for 8-gingerol, from 0.1 to 0.53 μg/mL for 10-gingerol and from 0.1 to 0.15 μg/mL for 6-shogaol, respectively.

3.3.2.2. Genotoxicity studies

In its assessment report on ginger rhizome, EMA considered genotoxicity studies of ginger rhizome inadequate and concluded that ginger rhizome has mutagenic as well as antimutagenic properties in microbial test systems (EMA, 2012). Particularly, 6-gingerol was identified as a powerful mutagen (Nakamura and Yamamoto, 1982 as referenced in EMA, 2012), whereas 6-shogaol was somewhat less active in *Salmonella*, but much less mutagenic in strain Hs30 of *Escherichia coli* than 6-gingerol. The authors of the study identified the hydroxylated aliphatic side chain moiety as the active part of 6-gingerol (Nakamura and Yamamoto, 1983, as referenced in EMA, 2012). The mutagenic potential of an ethanolic extract of ginger rhizome was investigated in Salmonella Typhimurium strains TA 100, TA 98, TA 1535 and TA 1538 (Nagabhushan et al., 1987, as referenced in EMA, 2012). The mutagenicity was restricted to the point mutation strains TA 100 and TA1535 with addition of a rat liver microsomal fraction. It could be demonstrated that the mutagenicity of the extract was due to the major components 6-gingerol and 6-shogaol, which both were strongly mutagenic in the bacterial test system. Zingerone, which was not mutagenic itself, inhibited the mutagenicity of 6-gingerol and 6-shogaol.

The genotoxicity of 6-gingerol was investigated *in vitro* in human hepatoma G2 (HepG2) cells (Yang et al., 2010). This hepatoma cell line retains the expression of Cyp450 enzymes and is often used as model for liver toxicity. The cells were treated in culture medium with 6-gingerol dissolved in DMSO. A dose-dependent increase of DNA-strand breaks investigated in the Comet assay was observed between 10 and 80 μM, being significant at ≥ 20 μM (5.9 μg/mL). Additionally, a dose-dependent increase of micronuclei was observed at concentrations between 10 and 40 μM (significant at ≥ 20 μM). The study also demonstrated that mitochondrial membrane destabilisation and release of lysosomal enzymes occur after treatment of the cells with 6-gingerol at higher doses (≥ 20 μM). Additionally, the formation of reactive oxygen species (ROS) and 8-OHdG, indicating an exposure of DNA to ROS, have been observed in the study at concentrations ≥ 20 μM. These findings suggest that the DNA damage observed is most probably the result of indirect effects associated with increased production of ROS, mitochondrial dysfunction and release of lysosomal enzymes leading to DNA strand breaks. In conclusion, 6-gingerol causes DNA damage in HepG2-human hepatoma cells in culture at concentration ≥ 20 μM (5.9 μg/mL) in association with increased ROS production and destabilisation of the mitochondrial membrane.

Gingerol(s) and shogaol(s) have not been individually investigated in *in vivo* genotoxicity studies. Several *in vivo* studies were performed with the aim to investigate the protective effects of ginger rhizome extracts on genotoxic effects induced by other compounds. In particular, in an *in vivo* study aimed at investigating the protective effects on bladder tumours induced by N-butyl-N-(4-hydroxybutyl)-nitrosamine and N-methyl-N-nitrosourea, mice were fed a diet containing a ginger extract (1% or 2% of a ginger rhizome extract containing 2.54% gingerols, resulting in 250 or 500 mg gingerols/kg feed) for 18 weeks (Bidinotto et al., 2006). The daily consumption of gingerols was approximately 75 mg/kg bw per day. Nor gross abnormalities or toxic symptoms were observed during the feeding or after the termination of the experiment. No DNA strand breaks (comet assay in peripheral leucocytes) were detected after weeks 1, 3, 10 and 18. Additionally, no micronuclei were observed after 18 weeks in peripheral blood samples. Overall the study showed that the oral application of a mixture of gingerols/shogaols up to a daily dose of 75 mg/kg bw was not genotoxic in mice with respect to the induction of DNA-strand breaks or micronuclei in peripheral blood cells. Another *in vivo* study investigated the anti-genotoxic properties of an aqueous ginger rhizome extract (content of gingerols not specified) on dimethylbenz(a)anthracene induced genotoxicity in rat bone marrow cells. The administration of 250 mg/kg and 500 mg/kg of the extract in the diet did not alter

the frequency of micronuclei and chromosomal aberrations compared to the control group. However, since the content of gingerols in the extract was not reported, the results of this study are of limited relevance for the present assessment (Rout et al., 2015).

The available data suggest that gingerol and shogaol induce DNA damage *in vitro*, as demonstrated in bacterial and mammalian cell systems. These effects occur at concentration $\geq$ 20 μM (5.9 μg/mL). No genotoxicity could be observed *in vivo* at oral doses of up to 75 mg/kg bw, most probably, because the effective concentration is not available systemically. Another reason may be that the parent gingerols do not appear in plasma. After absorption, the compounds are conjugated with glucuronic acid or sulfate, which are hydrophilic and thus, less available in the cytoplasm of target cells. It can be assumed that the DNA damage observed *in vitro* is a result of oxidative stress caused by formation of ROS occurring at higher concentrations, which will not be reached *in vivo*. Thus, the FEEDAP Panel concludes that the use of the additive in feed is unlikely to pose a genotoxic concern.

3.3.2.3. Repeated dose toxicity studies

Repeated dose toxicity studies with the ginger oleoresin under assessment were not submitted. The applicant identified two repeated dose toxicity studies with ginger rhizome powder or extract. Although the test materials used in the studies are different compared to the oleoresin under application, the Panel considers these studies relevant for the present assessment as the content of gingerols and shogaols was determined in both test items allowing a comparison with the additive under assessment.

In a repeated dose toxicity study (Rong et al., 2009), a total of 40 male and female rats (5 animals/sex per group) were treated by gavage with ginger rhizome powder suspended in 5% gum arabic at the dosages of 0, 500, 1,000 and 2,000 mg/kg bw per day for 35 days. The administration of ginger rhizome was not associated with any mortalities and abnormalities in general conditions, behaviour, growth and food and water consumption. Except for a dose-related decrease in serum lactate dehydrogenase activity in males, ginger rhizome did not induce changes of haematological or blood biochemical parameters compared to control animals. In general, ginger rhizome treatment caused no macroscopically visible organ abnormality. Only at the highest dose (2,000 mg/kg), absolute and relative weights of testes were reduced (by 14.4% and 11.5%, respectively). From this study, an NOAEL of 1,000 mg/kg bw of ginger rhizome powder was estimated. According to the authors, the ginger rhizome powder contains 9.1 mg/kg of 6-gingerol and 1.6 mg/kg of 6-shogaol. From these values, an NOAEL of 11 mg/kg bw can be calculated for 6-gingerol + 6-shogaol. The Panel considered that the study is adequate to derive an NOAEL while acknowledging the uncertainty arising from its duration and incomplete pathology. Moreover, the absorption of compounds from a powder prepared from whole rhizome material may be less than that from an extract, such as the oleoresin, because of matrix effects.

The second study was aimed at evaluating the possible teratogenic potential of a ginger rhizome ethanol extract in rats. The study was performed in accordance with the OECD codes of Good Laboratory Practices (GLP). (Weidner and Siegwart, 2001). The extract was administered by oral gavage in doses of 0 (vehicle: sesame oil), 100, 333 and 1,000 mg/kg, to three groups of 22 pregnant female rats from days 6 to 15 of gestation. Body weight and food and water intake were recorded during the treatment period. On day 21 of gestation, the rats were killed and examined for standard parameters of reproductive performance. The foetuses were examined for signs of teratogenic and toxic effects. No mortality or adverse treatment-related effects were observed. The extract was well tolerated. No differences were observed in weight gain, food consumption and reproductive parameters. When foetuses were examined for external, visceral and skeletal changes, there were no signs of developmental toxicity. The authors of the study concluded that ginger rhizome extract administered to pregnant rats during the period of organogenesis, caused neither maternal nor developmental toxicity at daily doses of up to 1,000 mg/kg bw, which was identified as the NOAEL of the extract. As the concentration of gingerols in the extract accounted for 1.9%, the calculated corresponding NOAEL for gingerols (mixture of 6-, 8-, and 10-gingerol) would be 19 mg/kg bw. As the study showed several shortcomings, i.e. only female rats were included, the treatment period was only 9 days and the study duration was only 21 days, blood parameters were not examined, it cannot be used to derive an NOAEL for any end-point other than fetotoxicity. The FEEDAP Panel considers the study as supporting evidence in the identification of a reference point for gingerols and shogaols.

Based on the available evidence for gingerols and shogaols, the FEEDAP Panel retains the NOAEL of 11 mg/kg bw per day identified in the 35-day toxicity study for the sum of gingerols and shogaols based on a reduction of absolute and relative weights of testes observed at the highest dose.

An NOAEL of 500 mg/kg bw per day (the highest dose tested) was identified for an essential oil from ginger rhizome from the 90-day oral toxicity rat study described in Section 3.3.2.2. Since the essential oil tested contains about 31% of α-zingiberene, the FEEDAP Panel derived an NOAEL of 155 mg α-zingiberene/kg bw per day from that study.

3.3.2.4. Safety for the target species

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

In the absence of these data, 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 (EFSA SC, 2019a).

As the additive under assessment is sufficiently characterised, the FEEDAP Panel applied a component-based approach to assess the safety for target species of the oleoresin.

Based on considerations related to structural and metabolic similarities, the components were allocated to eight assessment groups, six of which correspond to the chemical groups (CGs) 31 (and related subgroups), 8 and 1, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 ('aliphatic and aromatic hydrocarbons'), the application of sub-assessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 is applied (EFSA CEF Panel, 2015a,b). The two remaining groups are constituted by zingiberene derivatives and by gingerols and shogaols. The allocation of the components to the (sub-)assessment groups is shown in Table 7.

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 oleoresin and the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017a). Default values on body weight are used to express exposure in terms of mg/kg bw. The intake levels of the individual components calculated for chickens for fattening, the species with the highest ratio of feed intake/body weight, are shown in Table 7.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification. For some components in the assessment group, toxicological data were available to derive no observed adverse effect level (NOAEL) values. Structural and metabolic similarity among the components in the assessment groups were assessed 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 repeated dose/subchronic studies, from which NOAEL values could be derived, were available for 6-gingerol + 6-shogaol, zingiberene (see Section 3.3.2.2), octyl acetate [09.007] in CG 1 (EFSA FEEDAP Panel, 2013), limonene [01.045], myrcene [01.008] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016a).

Considering the structural and metabolic similarities between gingerols and shogaols, read-across was applied using the NOAEL of 11 mg/kg bw per day for 6-gingerol + 6-shogaol to extrapolate to all the other 8- and 10-derivatives, and the NOAEL of 155 mg/kg bw per day for zingiberene [02.013] to extrapolate to ar-curcumene and vanillyl acetone.

In CG 31, the NOAELs for the representative compounds myrcene [01.008], limonene [01.045] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment group II (α-farnesene [01.140]), group III (β-sesquiphellandrene, β-bisabolene [01.028] and β-phellandrene) and group V (β-pinene [01.003], α-pinene [01.004], camphene [01.009], δ-cadinene) (EFSA CEF Panel, 2015a,b).

Read-across was also applied using the NOAEL of 345 mg/kg bw per day for octyl acetate [09.007] to hexadecanoic acid [08.014] and selected as the reference point for CG 1.

For the remaining compounds, namely δ-germacrene [01.042] and isoborneol [02.059], toxicity studies performed with the compounds under assessment were not available and read-across was not possible. Therefore, the threshold of toxicological concern (TTC) approach was applied (EFSA FEEDAP Panel, 2012a, 2017a). The two compounds were allocated to Cramer class I.

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 mg/kg bw per day for Cramer Class I compounds). Reference points selected for each compound are shown in Table 7.

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, 2019a). An MOET > 100 allowed for interspecies- and intra-individual variability (as in the default 10 × 10 uncertainty factor).

The approach to the safety assessment of ginger oleoresin for the target species is summarised in Table 7. As the calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 20 mg/kg, the same conclusion can be extended to all animal species, except veal calves, cattle for fattening and dairy cows, for which a higher use level is proposed (150 mg/kg).

Table 7: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of ginger oleoresin classified according to assessment groups[(a)]

Tincture composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS-No**	**Max conc. in the oleoresin**	**Max Feed conc.**	**Intake**	**Cramer Class**	**NOAEL[(b)]**	**MOE**	**MOET**
Constituent	–	% (w/w)	mg/kg	mg/kg bw	–	mg/kg bw	–	–
Gingerols and shogaols								
6-Gingerol	–	4.21	0.704	0.056	II	11	197,81	
8-Gingerol	–	1.25	0.209	0.017	II	11	666,22	
10-Gingerol		2.1	0.351	0.028	II	11	396,56	
6-Shogaol	–	3.98	0.665	0.053	I	11	209,24	
8-Shogaol	–	0.89	0.149	0.012	I	11	935,71	
10-Shogaol		3.94	0.659	0.052	I	11	211,37	
MOET gingerols/ shogaols								51
Essential oil[(c)]								
Compounds no CGs								
Zingiberene	–	7.23	1.209	0.096	I	**155[(d)]**	1,628	
ar-Curcumene	–	2.01	0.336	0.027	I	155	5,838	
Zingerone	–	0.46	0.077	0.006	I	155	25,510	
MOET								1,213
CG 31, II								
α-Farnesene	01.040	1.70	1.209	0.096	I	44	1,959	
CG 31, III								
β-Sesquiphellandrene	–	3.45	0.577	0.046	I	250	5,486	
β-Bisabolene	01.028	1.83	0.306	0.024	I	250	10,343	
β-Phellandrene	01.055	2.13	0.356	0.028	I	250	8,886	
d-Limonene	01.045	3.93	0.657	0.052	I	**250**	4,816	
MOET CG 31, III								1,669
CG 31, V								
Camphene	01.009	4.51	0.754	0.060	I	222	3,726	
Pin-2(3)-ene	01.004	2.08	0.416	0.033	I	222	6,755	
Pin-2(10)-ene	01.003	1.53	0.306	0.024	I	222	9,183	
δ-Cadinene	–	1.00	0.200	0.016	I	222	14,051	
MOET CG 31, V								1,677

Tincture composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS-No**	**Max conc. in the oleoresin**	**Max Feed conc.**	**Intake**	**Cramer Class**	**NOAEL[(b)]**	**MOE**	**MOET**
CG 31, VI								
Germacrene D	01.042	1.02	0.171	0.013	I	*3*	223	
CG 1								
Hexadecanoic acid	08.014	0.24	0.055	0.004	I	**120**	27,530	
CG 8								
Isoborneol	02.059	0.46	0.077	0.006	I	*3*	493	

FLAVIS No: EU Flavour Information System number; NOAEL: no observed adverse effect level; MOE: margin of exposure; MOET: combined margin of exposure.

(a): Intake calculations for the individual components are based on the use level of 20 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 sum of the reciprocals of the MOE of the individual substances

(b): 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.

(c): Individual components of essential oil classified according to assessment groups based on chemical groups (CGs) as defined in Annex I of Regulation (EC) No 1565/2000

(d): NOAEL derived from the 90-day study with the essential oil.

More than 10 components detected in the volatile fraction remained unidentified. Taken together, they represent 0.12% (0–0.17%) of the oleoresin and would lead to a maximum of 0.028 mg/kg feed. The largest unidentified compound in this fraction (0.1% of the oleoresin) would lead to 0.016 mg/kg feed, which would be below the threshold for Cramer Class I, II and III compounds. Therefore, the FEEDAP Panel considers it unlikely that this compound and the other unidentified compounds in the volatile fraction would be of concern.

As shown in Table 7, the MOET was > 100 for all the assessment groups, except for the sum of gingerols and shogaols, for which the calculated MOET is 51. The FEEDAP Panel considers that, based on the data set available for gingerols and shogaols, the magnitude of the MOET is not wide enough to exclude a safety concern from the use of ginger oleoresin as a feed additive at the proposed use level (20 mg/kg complete feed). Gingerols and shogaols are, therefore, the compounds limiting the safety assessment of ginger oleoresin.

Since for this assessment group the safety of the proposed use level cannot be demonstrated based on the toxicological data set available, the FEEDAP Panel applies the approach based on the NOAEL to calculate safe concentrations in feed for the sum of gingerols and shogaols, as described in the EFSA guidance on the safety for target species (EFSA FEEDAP Panel, 2017a). For the components of the assessment group 'gingerols and shogaols', the FEEDAP Panel considers the NOAEL of 11 mg/kg bw per day derived from the 35-day study with ginger rhizome powder as a suitable reference point to assess the safety of the ginger oleoresin under assessment. However, because of the shorter duration of the study (35 instead of 90 days) and the differences in the test material (ginger rhizome powder instead of oleoresin), the FEEDAP Panel considers it appropriate to increase the default UF of 100 for extrapolation from the rat experiment to other species by an additional factor of 2.

Applying an uncertainty factor (UF) of 200 to the NOAEL, the safe daily dose for the target species was derived following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), and thus, the maximum safe feed concentration of gingerols and shogaols was calculated (Table 8). From this concentration and considering that the sum of gingerols and shogaols could represent up to 13.1% of the oleoresin (Table 6), the maximum safe concentration of the oleoresin in feed is calculated.

Feline species have a genetic deficiency in glucuronyltransferases (see Section 3.3.1.1). Experiments described in Section 3.3.2.1 have shown that glucuronidation is an important metabolising step of gingerols and shogaols. The UF for cats has, therefore, to be increased by a factor of 5. Because the UF was already increased by a factor of 2, an additional UF of 2.5 is considered appropriate.

Table 8: Maximum safe concentration in feed for different target animals for gingerols and shogaols and for ginger oleoresin

	Body weight (kg)	Feed intake (g DM/day)	Daily feed intake (g DM/kg bw)	Maximum safe concentration gingerols+shogaols (mg/kg feed)(1)	Maximum safe concentration oleoresin(2) (mg/kg feed)(1)
Chickens for fattening	2	158	79	0.6	4.7
Laying hens	2	106	53	0.9	7.0
Turkeys for fattening	3	176	59	0.8	6.3
Piglets	20	880	44	1.1	8.4
Pigs for fattening	60	2,200	37	1.3	10.1
Sow lactating	175	5,280	30	1.7	13.2
Veal calves (milk replacer)	100	1,890	19	2.8	21.0
Cattle for fattening	400	8,000	20	2.4	18.5
Dairy cows	650	20,000	31	1.6	12.0
Sheep/goat	60	1,200	20	2.4	18.5
Horse	400	8,000	20	2.4	18.5
Rabbit	2	100	50	1.0	7.4
Salmon	0.12	2.1	18	2.8	21.1
Dogs	15	250	17	2.9	22.2
Cats(3)	3	60	20	1.0	7.4
Ornamental fish	0.012	0.054	5	10.8	82.1

(1): Complete feed containing 88% DM, milk replacer 94.5% DM.
(2): Calculated by dividing the maximum safe concentrations of gingerols and shogaols in feed by the maximum percentage of the sum of gingerols and shogaols in the oleoresin (13.1%).
(3): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

Based on the toxicity of the identified components in the ginger oleoresin, the maximum proposed feed concentration of 20 mg/kg complete feed is safe for fish, sheep, goats and horses, and the maximum proposed feed concentration of 1 mg/kg is safe for cats and dogs and other pets. For the remaining species, the calculated maximum safe concentration of ginger oleoresin in feed is 5 mg/kg complete feed for chickens for fattening, 7 mg/kg for laying hens, 6 mg/kg for turkeys for fattening, 8 mg/kg for piglets, 10 mg/kg for pigs for fattening, 13 mg/kg for sows, 12 mg/kg for dairy cows, 21 mg/kg for veal calves, 19 mg/kg for cattle for fattening and 7 mg/kg for rabbits. At these concentrations in feed, the volatile components of ginger oleoresin do not raise concern.

The same conclusion would apply if the oleoresin is used in water for drinking at comparable exposure.

3.3.2.5. Safety for the consumer

Rhizomes of *Z. officinale* and their preparations including the oleoresin are added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2010) cites values of 3 mg/kg bw per day for ginger rhizomes and of 0.025 mg/kg bw per day for ginger oleoresin.

No data on residues in products of animal origin were made available for any of the constituents of ginger oleoresin. When considering the ADME of the individual components, gingerols and shogaols (see Section 3.3.2.1) as well as the volatile components of the essential oil, which show a rapid conjugation and elimination (see Sections 3.3.2.1 and 3.2.2.1.), a relevant increase of the uptake of these compounds by humans consuming products of animal origin is not expected.

Considering the reported human exposure due to direct use of ginger rhizomes and its preparations in food (Burdock, 2010), it is unlikely that consumption of products from animals given ginger oleoresin at the proposed maximum use level would significantly increase human background exposure.

Consequently, no safety concern would be expected for the consumer from the use of ginger oleoresin up to the highest safe use level in feed.

3.3.2.6. Safety for the user

No specific data were provided by the applicant regarding the safety of the oleoresin for users.

6-Gingerol is classified by ECHA CLP as skin irritant (H315), eye irritant (H 319) and respiratory irritant (H335). Other gingerols and shogaols are expected to show the same characteristics.

Ginger oil has been notified to ECHA for classification according to CLP as aspiratory toxic (H304), skin irritant (H315), skin sensitiser (H317), eye irritant (H319) and respiratory irritant (H335).

3.3.2.7. Safety for the environment

There are no data on the safety of ginger oleoresin for the environment.

The active substances (gingerols and shogaols) are phenols that are excreted mainly as conjugates (glucuronides, sulfates) and/or after metabolisation by ω-oxidation and β-oxidation of the phenolic side chain.

Seven of the identified constituents of the essential oil (camphene, D-limonene, β-phellandrene, pin-2(3)-ene, pin-2(10)-ene, isoborneol and hexadecanoic acid) have been evaluated by EFSA as sensory additives for animal feed, they were considered to be safe for the environment at use individual levels higher than those resulting from the use of the oleoresin in feed.

The remaining identified major constituents of the essential oil are mainly aliphatic mono or sesquiterpenes partially with functional groups, they are chemically related to the substances evaluated by EFSA as CG 31 for use in animal feed (EFSA FEEDAP Panel, 2015, 2016a) for which EFSA concluded that they were 'extensively metabolised by the target species (see Section 3.2.1) and excreted as innocuous metabolites or carbon dioxide'. Therefore, no risk for the safety for the environment is foreseen. Average feed levels of constituents of the essential oil are much lower than the use levels for CG 31 substances. Zingiberenol, a minor constituent of the essential oil, is present in feed at levels below any environmental concern.

The non-identified constituents of the essential oil of ginger oleoresin are chemically related to the identified ones and the assessment presented above will apply to those as well. It should be noted that the largest unidentified constituent results in feed levels below 0.04 mg/kg; therefore, the expected PEC_{soil} will be far below the trigger.

The use of ginger oleoresin in feed is not expected to pose a risk for the environment.

3.4. Ginger tincture

Ginger tincture is obtained by extraction of ground dried rhizomes of *Z. officinale* using an ethanol/water mixture (90/10). The ratio of dry raw material to solvent is 1:4 (w:v). Following maceration for 21 days, the tincture is obtained by pressing to remove solid material, and filtration.[28] Besides soluble carbohydrates, lipids and proteins, the dry matter (DM) fraction of the tincture contains gingerols (6-, 8- and 10-gingerol) and shogaols (6- and 8-shogaol), volatile components from the essential oil and phenols other than gingerols and shogaols.

3.4.1. Characterisation

The tincture is a colourless to yellowish liquid with characteristic odour of ginger rhizomes having a spicy flavour. It has a density of 827–844 kg/m^3 (836 kg/m^3 on average). The product is an ethanol/water (90/10) solution, which contains by specification 800–1,300 μg/mL of total gingerols and 200–400 μg/mL of total shogaols.[29]

Table 9 summarises the results of proximate analysis of five batches of the additive (from Chinese origin) expressed as % (w/w).[30] The solvent represents up to 98% of the additive, the DM content ranged between 1.75 and 2.38 g/100 mL (average 1.95 g/100 mL).[31]

[28] Technical dossier/Supplementary information August 2018/Annex_II_Ging_Tinct_Production_protocols.
[29] Technical dossier/Supplementary information August 2018.
[30] Technical dossier/Supplementary information August 2018/Annex_VI_Ging_Tinct_Nutr_Microbiol.
[31] Technical dossier/Supplementary information August 2018/Annex_V_Ging_Tinct_GRavit_Anal_PCDD-PCDFF.

Table 9: Proximate analysis of ginger tincture (*Zingiber officinale* Roscoe) based on the analysis of five batches (mean and range). The results are expressed as % (w/w)

Constituent	Mean[(a)]	Range
	% (w/w)	% (w/w)
Dry matter	2.34	2.08–2.88
Ash	0.1	< 0.1–0.1
Total sugars	0.5	< 0.5–0.5
Lipids	0.56	0.5–0.7
Protein	0.1	< 0.1–0.1
Fibre	< 0.5	< 0.5
Solvent (ethanol/water, 90/10)	97.66	97.12–97.92

(a): Mean calculated on five batches.

The fraction of secondary metabolites was characterised in the same batches of the additive and the results are summarised in Table 10. Individual gingerols and shogaols were determined by HPLC with UV detector,[32] the essential oil fraction was characterised by gas chromatography coupled with a flame ionisation detector (GC-FID) and mass spectrometry (GC-MS).[33] Phenols determined by spectrophotometry (at 760 nm) are expressed as gallic acid equivalents.[34] Analytical results are expressed as μg/mL. With respect to the secondary metabolites, the tincture contains on average 4,958 μg/mL volatile compounds (corresponding to 0.59% (w/w), when considering the average density of the tincture 836 kg/m^3) and 2,132 μg/mL phenols (0.26% (w/w)), including 1,085 μg/mL gingerols (0.13% (w/w)) and 299 μg/mL shogaols (0.036% (w/w)). The corresponding figures for the maximum concentrations are 8,242 μg/mL (0.99% (w/w)) volatile compounds, 2,281 μg/mL phenols (0.28% (w/w)), including 1,161 μg/mL gingerols (0.14% (w/w)) and 356 μg/mL shogaols (0.043% (w/w)). The fraction of secondary metabolites including volatiles accounts on average for 36% of the dry matter fraction of the tincture (range: 24%–59%) and the other plant constituents for about 60%.[35]

Table 10: Characterisation of the fraction of secondary metabolites (including volatiles) of ginger tincture (*Zingiber officinale* Roscoe) based on the analysis of five batches (mean and range). The results are expressed as μg/mL of ginger tincture

Constituent	CAS No	FLAVIS No	Mean[(a)]	Range
			μg/mL	μg/mL
Phenols (total, by photometry)	–	–	2,132	1,952–2,281
Gingerols (total, HPLC)			1,085	941–1,161
6-Gingerol	23513-14-6	–	835	714–936
8-Gingerol	23513-08-8	–	212	183–257
10-Gingerol	23513-15-7	–	43.6	22.3–53.3
Shogaols (total)			299	262–356
6-Shogaol	555-66-8	–	255	216–285
8-Shogaol	104186-07-4	–	39.3	16.5–50.6
Essential oil (GC-FID)			4,958	2,330–8,242
Camphene	79-92-5	01.009	111	4–509
β-Phellandrene	555-10-2	01.055	153.6	9–691
1,8 Cineole	470-82-6	03.001	29	6–92
D,L-Borneol	507-70-0	02.016	35	21–50
α-Terpineol	98-55-5	02.014	14.8	9–20

[32] Technical dossier/Supplementary information August 2018/Annex_III_Ging_Tinct_Certificates of analysis.
[33] Technical dossier/Supplementary information August 2018/Annex_VII_Ging_Tinct_GC-MS GC-FID ess Oils.
[34] Technical dossier/Supplementary information August 2018/Annex_VIII_Total_Phenols.
[35] Average of 36% calculated summing the fractions (expressed as %, w/w) of phenols (0.26%) and volatiles (0.59%) and dividing for the DM (1.34%, w/w). The range (24–59%) is obtained by dividing the sum of phenols and volatiles calculated for each individual batch by the corresponding value of the DM in the same batch

Constituent	CAS No	FLAVIS No	Mean[(a)]	Range
			µg/mL	µg/mL
Geraniol	106-24-1	02.012	12.6	11–15
(E)-Citral	141-27-5	05.188	19.4	6–52
ar-Curcumene	644-30-4	–	625.2	309–1,040
Germacrene D	23986-74-5	01.042	10.2	5–15
Zingiberene	495-60-3	–	1454	576–2,135
α-Farnesene	502-61-4	01.040	234	103–319
β-Bisabolene	495-61-4	01.028	564	253–855
β-Sesquiphellandrene	20307-83-9	–	690	356–979
Zingiberenol	58334-55-7	–	50.8	34–63
Total unidentified			958	588–1,542

(a): Mean calculated on five batches.

3.4.1.1. Impurities

Data on impurities were provided for three batches of ginger tincture. The concentrations of heavy metals and arsenic were below the corresponding LOQ, with the exception of arsenic (0.01 mg/kg) and lead (0.02 mg/kg) in one batch. In the same batches, mycotoxins (aflatoxins B1, B2, G1 and G2) were below the LOQ and pesticides were not detected in a multiresidue analysis.[36] When specifically analysed, biphenyl (0.055–0.060 mg/L) and diethyltoluamide (DEET, 0.06–0.11 mg/L) were detected in all three batches. Dioxin-like polychlorinated biphenyls (PCBs) ranged between 0.51 and 1.25 pg/L. The sum of dioxins was in the range 9.97–25.27 ng WHO PCDD/F-TEQ (World Health Organisation polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) toxic equivalents)/kg.[37] None of the data on chemical impurities raised concerns.

Analysis of microbial contamination of five batches of ginger tincture indicated that *Salmonella* spp. were absent in 25 g, *E. coli* and *Enterobacteriaceae* were $< 10^1$ colony-forming unit (CFU)/g.[30]

3.4.1.2. Shelf-life

The applicant states that the typical shelf-life of flavourings is at least 12 months, when stored in tightly closed containers under standard conditions. No stability studies were performed for the tincture.

3.4.1.3. Conditions of use

Ginger tincture is intended to be added to water for drinking for poultry at a maximum proposed use levels of the tincture are 0.9 mL/L water for drinking (corresponding to 1.8 mL/kg complete feed). It is also intended for use in complete feed for horses and dogs at a maximum use level of 1.6 mL/kg and 0.17 mL/kg, respectively.

3.4.2. Safety

The assessment of safety is based on the maximum use levels proposed by the applicant: 0.9 mL/L water for drinking or 1.8 mL/kg complete feed (which corresponds to 1.50 g/kg complete feed, considering the average density of the additive of 836 kg/m^3) for poultry, 1.6 mL/kg complete feed for horses and 0.17 mL/kg complete feed for dogs.

The ADME of the individual components of ginger tincture has been already described in Section 3.2.2.1 (components of ginger essential oil) and in Section 3.3.2.1 (gingerols and shogaols).

Toxicological studies with the ginger tincture under assessment are not available to the FEEDAP Panel. The studies relevant to the assessment of the individual components of ginger tincture have been already described in Sections 3.2.2.2 and 3.3.2.2.

[36] Technical dossier/Supplementary information August 2018/Annex_X_Ging_Tinct_Impurities. LOQ for heavy metals and arsencic: < 0.01 mg/kg for lead and arsenic, < 0.002 mg/kg for mercury and cadmium; LOQ for individual pesticides: 0.001–0.005 mg/L; LOQ for mycotoxins: < 0.1 µg/kg for aflatoxins B1, B2, G1 and G2 and ochratoxin A, < 2 µg/kg for zearalenon, HT2-toxin and T2-toxin, < 5 µg/kg for nivalenol and < 10 for deoxynivalenol.

[37] Technical dossier/Supplementary information August 2018/Annex_V_Tinct_GRavit_Anal_PCDD-PCDFF.

3.4.2.1. Safety for the target species

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

In the absence of these data, 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, EFSA SC, 2019a).

The tincture consists of 98% of a water/ethanol mixture. The concentration of plant-derived compounds is about 2% of the tincture, of which 1.76% was identified as ash, protein, lipids, carbohydrates and fibre. These components identified by the proximate analysis are not of concern and are not further considered. Among the identified secondary metabolites, 0.6% is volatile, 0.26% is phenolic in nature and 0.17% is constituted by gingerols and shogaols (which are also phenolic compounds). The concentration of unidentified compounds in the tincture is < 0.5%.

The approach to the safety assessment of ginger tincture for the target species follows the principles described in Section 3.3.2.3 for the oleoresin and is summarised in Table 9. Based on considerations related to structural and metabolic similarities, the components were allocated to several assessment groups: gingerol and shogaols and volatile components from the essential oil.

Toxicological data, from which NOAEL values could be derived, were available for 6-gingerol + 6-shogaol, zingiberene (see Section 3.3.2.2), limonene [01.045], myrcene [01.008] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016a), 1,8 cineole [03.001] in CG 16 (EFSA FEEDAP Panel, 2012d), α-terpineol [02.014] in CG 6 (EFSA FEEDAP Panel, 2012e) and citral [05.188] in CG 3 (EFSA FEEDAP Panel, 2016b).

In CG 31, the NOAELs for the representative compounds myrcene [01.008], limonene [01.045] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment group II (α-farnesene [01.140]), group III (β-sesquiphellandrene, β-bisabolene [01.028] and β-phellandrene) and group V (camphene [01.009], δ-cadinene) (EFSA CEF Panel, 2015a,b).

Read-across was also applied using the NOAEL of 345 mg/kg bw per day for citral [09.007] to geranial [02.012] and selected as the reference point for CG 3.

For the remaining compounds, namely δ-germacrene [01.042] and borneol [02.016], toxicity studies and NOAEL values performed with the compounds under assessment were not available and read-across was not possible. Therefore, the threshold of toxicological concern (TTC) approach was applied (EFSA FEEDAP Panel, 2012a, 2017a). The two compounds belong to Cramer class I.

For each assessment group, the combined (total) margin of exposure (MOET) was calculated.

Table 11: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of ginger tincture classified according to assessment groups[(a)]

Tincture composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS-No**	**Max conc. in the tincture**	**Max Feed conc.**	**Intake**	**Cramer Class**	**NOAEL[(b)]**	**MOE**	**MOET**
Constituent	–	% (w/w)	mg/kg	mg/kg bw	–	mg/kg bw	–	–
Gingerols and shogaols								
6-Gingerol	–	0.113	1.703	0.135	II	11	81.48	
8-Gingerol	–	0.027	0.406	0.032	II	11	343.75	
10-Gingerol		0.020	0.295	0.023	II	11	478.26	
6-Shogaol	–	0.035	0.519	0.041	I	11	268.29	
8-Shogaol	–	0.006	0.092	0.007	I	11	1,571.43	
MOET gingerols/ shogaols								46
Essential oil[(c)]		0.992	14.925	1.179				
Compounds no CGs								
Zingiberene	–	0.257	3.866	0.305	I	**155[(d)]**	509	
ar-Curcumene	–	0.117	1.766	0.139	I	155	1,111	
Zingiberenol	–	0.008	0.115	0.009	I	155	17,116	

Tincture composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS-No**	**Max conc. in the tincture**	**Max Feed conc.**	**Intake**	**Cramer Class**	**NOAEL[(b)]**	**MOE**	**MOET**
MOET								342
CG 31, II								
α-Farnesene	01.040	0.038	0.578	0.046	I	44	964	
CG 31, III								
β-Sesquiphellandrene	–	0.118	1.781	0.141	I	250	1,776	
β-Bisabolene	01.028	0.103	1.556	0.123	I	250	2,034	
β-Phellandrene	01.055	0.083	1.251	0.099	I	250	2,529	
MOET CG 31, III								690
CG 31, V								
Camphene	01.009	0.061	0.922	0.073	I	222	3,049	
CG 31, VI								
Germacrene D	01.042	0.002	0.027	0.002	I	*3*	1,398	
CG 3								
Geraniol	02.012	0.002	0.027	0.002	I	345	163,292	
(E)-Citral	05.188	0.006	0.094	0.007	I	**345**	46,378	
MOET CG 3								36,119
CG 6								
α-Terpineol	02.014	0.002	0.036	0.003	I	**250**	87,379	
CG 8								
Borneol	02.016	0.006	0.091	0.007	I	*3*	419	
CG 16								
1,8-Cineole	03.001	0.011	0.167	0.013	II	**562.5**	42,740	

FLAVIS No: EU Flavour Information System number; NOAEL: no observed adverse effect level; MOE: margin of exposure; MOET: combined margin of exposure.

(a): Intake calculations for the individual components are based on the use level of 1.8 mL/kg (corresponding to 1.5 g/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 sum of the reciprocals of the MOE of the individual substances.

(b): 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.

(c): individual components of essential oil classified according to assessment groups based on chemical groups (CGs) as defined in Annex I of Regulation (EC) No 1565/2000

(d): NOAEL derived from the 90-day study with the essential oil.

More than 50 components detected in the volatile fraction remained unidentified. Taken together, they represent 0.11% of the tincture and would lead to maximum of 1.65 mg/kg feed. The largest unidentified compound of the volatile fraction (0.012% of the tincture) would lead to 0.18 mg/kg feed, which would be below the threshold for Cramer Class I compounds but above the thresholds for Cramer class III and II. However, the analysis of the essential oil showed that the majority of compounds belong to Class I and a literature survey did not identify compounds of concern in extracts from dried rhizomes of *Z. officinale*. Therefore, the FEEDAP Panel considers it unlikely that this compound and the other unidentified compounds in the volatile fraction would be of concern.

As shown in Table 11, the MOET was > 100 for all the assessment groups, except for the sum of gingerols and shogaols, for which the calculated MOET of 46 is considered not wide enough to exclude a safety concern from the use of ginger tincture as feed additive at the proposed use level (1.8 mL/kg complete feed for poultry). Gingerols and shogaols are, therefore, the compounds limiting the safety assessment of ginger oleoresin.

Since for this assessment group the proposed use level will be unsafe, the FEEDAP Panel would apply the approach based on the NOAEL to calculate safe concentrations in feed for the sum of

gingerols and shogaols, as described in the EFSA guidance on the safety for target species (EFSA FEEDAP Panel, 2017a). Applying an UF of 200 to the NOAEL of 11 mg/kg bw per day from the 35-day study with ginger rhizome powder, the safe daily dose for the target species was derived following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), and thus, the maximum safe feed concentration of gingerols and shogaols was calculated (Table 12). From this concentration and considering that the sum of gingerols and shogaols could represent up to 0.18% of the tincture (Table 8), the maximum safe concentration of the tincture in feed is calculated.

Table 12: Maximum safe concentration in feed for different target animals for gingerols and shogaols and for ginger tincture

	Body weight (kg)	Feed intake (g DM/day)	Daily feed intake (g DM/kg bw)	Maximum safe concentration gingerols+shogaols (mg/kg feed)(1)	Maximum safe concentration tincture(2) (mL/kg feed)(1)
Chickens for fattening	2	158	79	0.6	0.40
Laying hens	2	106	53	0.9	0.60
Turkeys for fattening	3	176	59	0.8	0.54
Horses	400	8,000	20	2.4	1.58
Dogs	15	250	17	2.9	1.81

(1): Complete feed containing 88% DM, milk replacer 94.5% DM.
(2): Calculated by dividing the maximum safe concentrations of gingerols and shogaols in feed by the maximum percentage of the sum of gingerols and shogaols in the tincture (0.183%) and considering the average density of the tincture (836 kg/m^3).

The maximum proposed concentrations of 1.6 mL/kg feed for horses and 0.17 mL/kg for dogs are of no concern. For poultry species, the calculated maximum safe dose is below the proposed use level and ranges between 0.4 and 0.6 mL/kg complete feed, which would correspond to about 0.2–0.3 mg/L water for drinking (see Table 10).

3.4.2.2. Safety for the consumer

Rhizomes of *Z. officinale* and their preparations including ethanolic extracts are added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2010) cites values of 3 mg/kg bw per day for ginger rhizomes and 0.0005 mg/kg bw per day for ethanolic extracts of ginger rhizomes.

No data on residues in products of animal origin were made available for any of the constituents of ginger tincture. When considering the ADME of the individual components, gingerols and shogaols as well as the volatile components of the essential oil, which show a rapid conjugation and elimination (see Sections 3.3.2.1 and 3.2.2.1, respectively), a relevant increase of the uptake of these compounds by humans consuming products of animal origin is not expected.

Considering the reported human exposure due to direct use of ginger rhizomes and its preparations in food (Burdock, 2010), it is unlikely that consumption of products from animals given ginger tincture at the proposed maximum use level would significantly increase human background exposure.

Consequently, no safety concern would be expected for the consumer from the use of ginger tincture up to the highest safe use level in feed.

3.4.2.3. Safety for the user

No specific data were provided by the applicant regarding the safety of the additive for users.

The additive contains 85–90% ethanol which is the main hazard present. Ginger tincture is classified by ECHA CLP as eye irritant (H 319). 6-Gingerol is classified by ECHA CLP as acute toxic (H301, H302, H312, H332), skin irritant (H315), eye irritant (H 319) and respiratory irritant (H335).

Ginger oil has been notified to ECHA for classification according to CLP as aspiratory toxic (H304), skin irritant (H315), skin sensitiser (H317), eye irritant (H319) and respiratory irritant (H335).

3.4.2.4. Safety for the environment

There are no data on the safety of ginger tincture for the environment.

Gingerols, the most abundant constituents in ginger tincture will be present in the drinking water of poultry and horses at very low levels (< 1 mg/kg feed) and are unlikely to present any environmental concern.

The use of ginger tincture up to the highest safe use level in poultry and horses is not expected to pose a risk for the environment.

3.5. Efficacy of ginger essential oil, ginger oleoresin and ginger tincture

Ginger rhizome and its extracts are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2010) and by FEMA with the reference numbers 2520 (ginger), 2521 (ginger extract), 2522 ginger oil and 2323 (ginger oleoresin).[38]

Since ginger rhizome and its extracts are recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

The FEEDAP Panel concludes that the three preparations under consideration, ginger essential oil, ginger oleoresin and ginger tincture from *Zingiber officinale* Roscoe are safe for the target species at the following use levels:

- ginger essential oil is safe for all animal species up to the maximum proposed use level of 20 mg/kg feed (or 20 mg/L water for drinking). The higher maximum use level of 80 mg/kg for veal calves is also considered safe for this species category
- ginger oleoresin is safe at the maximum proposed concentration of 20 mg/kg complete feed for fish, sheep, goats and horses, and at the maximum proposed feed concentration of 1 mg/kg for cats and dogs and other pets. For the remaining species, the calculated maximum safe concentration of ginger oleoresin in feed is 5 mg/kg complete feed for chickens for fattening, 7 mg/kg for laying hens, 6 mg/kg for turkeys for fattening, 8 mg/kg for piglets, 10 mg/kg for pigs for fattening, 13 mg/kg for sows, 12 mg/kg for dairy cows, 21 mg/kg for veal calves, 19 mg/kg for cattle for fattening and 7 mg/kg for rabbits. The same conclusion would apply if the oleoresin is used in water for drinking at comparable exposure.
- ginger tincture is safe at the maximum proposed concentrations of 1.6 mL/kg feed for horses and 0.17 mL/kg for dogs. For poultry species, the calculated maximum safe dose is below the proposed use level and ranges between 0.2 and 0.3 mg/L water for drinking

No concerns for consumers were identified following the use of ginger essential oil, ginger oleoresin and ginger tincture up to the highest safe level in feed.

Ginger essential oil, ginger oleoresin and ginger tincture should be considered as irritants to skin and eyes and the respiratory tract and as a skin sensitisers.

The use of ginger essential oil, ginger oleoresin and ginger tincture in feed is not expected to pose a risk for the environment.

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

5. Recommendations

The FEEDAP Panel recommends that the authorisation should apply only to the preparations obtained from dried rhizomes of *Zingiber officinale* Roscoe.

If the essential oil is used simultaneously in feed and water for drinking, overdosage should be avoided.

Documentation provided to EFSA/Chronology

Date	Event
05/11/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 09 - Zingiberales 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

[38] Technical dossier/Section IV/ Page 5.

Date	Event
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
17/10/2012	Reception of supplementary information from the applicant
26/02/2013	EFSA informed the applicant (EFSA ref. 7150727) 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
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
12/05/2016	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". Discussion on the ongoing work regarding the pilot dossiers BDG08 and BDG 09
17/06/2016	Spontaneous submission of information by the applicant. *Issues: characterisation*
27/04/2017	Trilateral meeting organised by the European Commission with EFSA and the applicant FEFANA on the assessment of botanical flavourings: characterisation, substances of toxicological concern present in the botanical extracts, feedback on the pilot dossiers
24/07/2017	EFSA informed the applicant that the evaluation process restarted.
12/10/2017	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*
29/05/2018	Reception of supplementary information from the applicant (partial submission)
10/08/2018	Reception of supplementary information from the applicant (partial submission)
27/02/2019	EFSA was informed about the withdrawal of the application on ginger extract
19/03/2019	Reception of supplementary information from the applicant
17/02/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment re-started
02/04/2020	Spontaneous submission of information by the applicant. *Issues: safety for the consumer*
07/05/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

BDG	Botanically defined group
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
DM	dry matter
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agencies
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour Extract Manufacturers Association
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FLAVIS	the EU Flavour Information System
FL-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry
ISO	International standard organisation
LOQ	limit of quantification

JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
MOET	combined margin of exposure (total)
NOAEL	no observed adverse effect level
PCBs	polychlorobiphenyls
PCDD/F	polychlorinated dibenzo-p-dioxins and dibenzofurans
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization