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Safety and efficacy of α,β-unsaturated straight-chain and branched-chain aliphatic primary alcohols, aldehydes, acids and esters belonging to chemical group 3 when used as flavourings for all animal species

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

Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of 43 compounds belonging to chemical group 3 (α,β-unsaturated straight-chain and branched-chain aliphatic primary alcohols, aldehydes, acids and esters) when used as feed flavourings for all animal species and categories. They are currently authorised as flavours in food. This opinion concerns 17 compounds from this group. The FEEDAP Panel established the following conclusions: geraniol [02.012] and citral [05.020] are safe for all target species at the proposed maximum use level of 25 mg/kg feed; farnesol [02.029], (*Z*)-nerol [02.058], geranyl acetate [09.011], geranyl butyrate [09.048], geranyl formate [09.076], geranyl propionate [09.128], neryl propionate [09.169], neryl formate [09.212], neryl acetate [09.213], neryl isobutyrate [09.424] and geranyl isobutyrate [09.431] are safe at the maximum proposed use level of 5 mg/kg feed for all target species; 2-methyl-2-pentenoic acid [08.055], (*2E*)-methylcrotonic acid [08.064], ethyl (*E,Z*)-deca-2,4-dienoate [09.260] and prenyl acetate [09.692] are safe at the proposed normal use levels of 1 mg/kg complete feed for all animal species. No safety concern would arise for the consumer from the use of these compounds up to the highest safe level in feeds. Hazards for skin and eye contact and respiratory exposure are recognised for the majority of the compounds under application. Most are classified as irritating to the respiratory system. The concentrations considered safe for the target species are unlikely to have detrimental effects on the terrestrial and fresh water environments. As all of the compounds under assessment are used in food as flavourings and their function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

Keywords: sensory additives, feed flavourings, α,β-unsaturated straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids/esters, chemical group 3, safety

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7, in addition, Article 10(2) of that Regulation also 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 of 43 substances (geraniol, hex-2-en-1-ol, 3,7,11-trimethyldodeca-2,6,10-trien-1-ol, nona-2,6-dien-1-ol, pent-2-en-1-ol, nerol, non-2(*cis*)-en-1-ol, tr-2, *cis*-6-nonadien-1-ol, citral (mixture of geranial and neral), 2-dodecenal, nona-2(*trans*),6(*cis*)-dienal, nona-2,4-dienal, *trans*-2-nonenal, hex-2(*trans*)-enal, 2,4-decadienal, 2,4-heptadienal, hepta-2,4-dienal, deca-2(*trans*),4(*trans*)-dienal, dodec-2(*trans*)-enal, hept-2(*trans*)-enal, non-2-enal, nona-2(*trans*),6(*trans*)-dienal, undec-2(*trans*)-enal, *trans*-2-octenal, *trans*-2-decenal, tr-2, tr-4-nonadienal, tr-2, tr-4-undecadienal, 2-methyl-2-pentenoic acid, 2-methylcrotonic acid, geranyl acetate, geranyl butyrate, geranyl formate, allyl heptanoate, geranyl propionate, neryl propionate, neryl formate, neryl acetate, allyl hexanoate, ethyldeca-2(*trans*),4(*cis*)-dienoate, hex-2(*trans*)-enyl acetate, hex-2-enyl butyrate, neryl isobutyrate, geranyl isobutyrate and prenyl acetate) belonging to chemical group (CG) 3, when used as a feed additive for all animal species (category: sensory additives; functional group: flavourings). CG 3 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[3] as 'α,β-unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters with esters containing α,β-unsaturated alcohol and acetal containing α,β-unsaturated alcohols or aldehydes. No aromatic or heteroaromatic moiety as a component of an ester or acetal'. During the course of the assessment, this application was split and the present opinion covers 17 out of the 43 substances under application (see Section 1.2).

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). During the course of the assessment, the applicant withdrew the application for the use of chemically defined flavourings in water for drinking.[4] 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 1 December 2010.

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 geraniol (EU Flavour Information System (FLAVIS) number) [02.012], 3,7,11-trimethyldodeca-2,6,10-trien-1-ol [02.029], nerol [02.058], citral (mixture of geranial and neral) [05.020], 2-methyl-2-pentenoic acid [08.055], 2-methylcrotonic acid [08.064], geranyl acetate [09.011], geranyl butyrate [09.048], geranyl formate [09.076], geranyl propionate [09.128], neryl propionate [09.169], neryl formate [09.212], neryl acetate [09.213], ethyldeca-2(*trans*),4(*cis*)-dienoate [09.260], neryl isobutyrate [09.424], geranyl isobutyrate [09.431] and prenyl acetate [09.692], when used under the proposed conditions of use (see Section 3.1.3).

[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] Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG), Avenue Louise 130A, B-1050, Brussels, Belgium.

[3] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8.

[4] On 10 March 2016, EFSA was informed by the European Commission on the withdrawal of the application for re-authorisation of chemically defined flavourings – use in water.

1.2. Additional information

Thirty-two out of the 43 compounds have been previously assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 1997, 1999, 2004, 2005). JECFA concluded that all flavouring substances evaluated were of no safety concern when used at current levels of estimated intake. A group acceptable daily intake (ADI) of 0.5 mg/kg body weight (bw) per day has been specified for geraniol, citral and geranyl acetate (WHO, 1980, 2004).

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has considered all 43 compounds, but could proceed with the assessment of only 17 compounds (EFSA, 2009a,b; EFSA CEF Panel, 2010a, 2013a,b), because of genotoxicity alerts based on the presence of α, β-unsaturated aldehydes and their precursors for the remaining 26 substances. The EFSA CEF Panel has requested additional data (EFSA CEF Panel, 2014a,b) and the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) will also not proceed with an assessment of these compounds until the issue of genotoxicity has been resolved.

The current assessment concerns 17 compounds, namely geraniol [02.012], 3,7,11-trimethyldodeca-2,6,10-trien-1-ol [02.029], nerol [02.058], citral (mixture of geranial and neral) [05.020], 2-methyl-2-pentenoic acid [08.055], 2-methylcrotonic acid [08.064], geranyl acetate [09.011], geranyl butyrate [09.048], geranyl formate [09.076], geranyl propionate [09.128], neryl propionate [09.169], neryl formate [09.212], neryl acetate [09.213], ethyldeca-2(*trans*),4(*cis*)-dienoate [09.260], neryl isobutyrate [09.424], geranyl isobutyrate [09.431] and prenyl acetate [09.692] (EFSA, 2009a,b; EFSA CEF Panel, 2010a, 2013a,b). All are currently listed in the European Union database of flavouring substances[5] and in the European Union Register of Feed Additives, and thus authorised for use in food and feed in the European Union (EU). They have not been previously assessed by EFSA as feed additives.

Regulation (EC) No 429/2008[6] allows substances already approved for use in human food to be assessed with a more limited procedure than for other feed additives. However, the use of this procedure is always subject to the condition that food safety assessment is relevant to the use in feed.

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 the compounds belonging to CG 3 as feed additives. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008 and the applicable EFSA guidance documents.

The FEEDAP Panel has sought to use 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 and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the 'α,β-unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols, aldehydes, acids, acetals and esters with esters containing α,β-unsaturated alcohol and acetal containing α,β-unsaturated alcohols or aldehydes. No aromatic or heteroaromatic moiety as a component of an ester or acetal in animal feed'. The Executive Summary of the EURL report can be found in Annex A.[8]

[5] 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.

[6] 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.

[7] FEED dossier reference: FAD-2010-0124.

[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FiniRep-FAD-2010-0124.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of 17 compounds belonging to CG 3, is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: 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 for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

3.1. Characterisation

3.1.1. Characterisation of the flavouring substances

The molecular structures of the 17 additives under assessment are shown in Figure 1 and their physicochemical characteristics in Table 1.

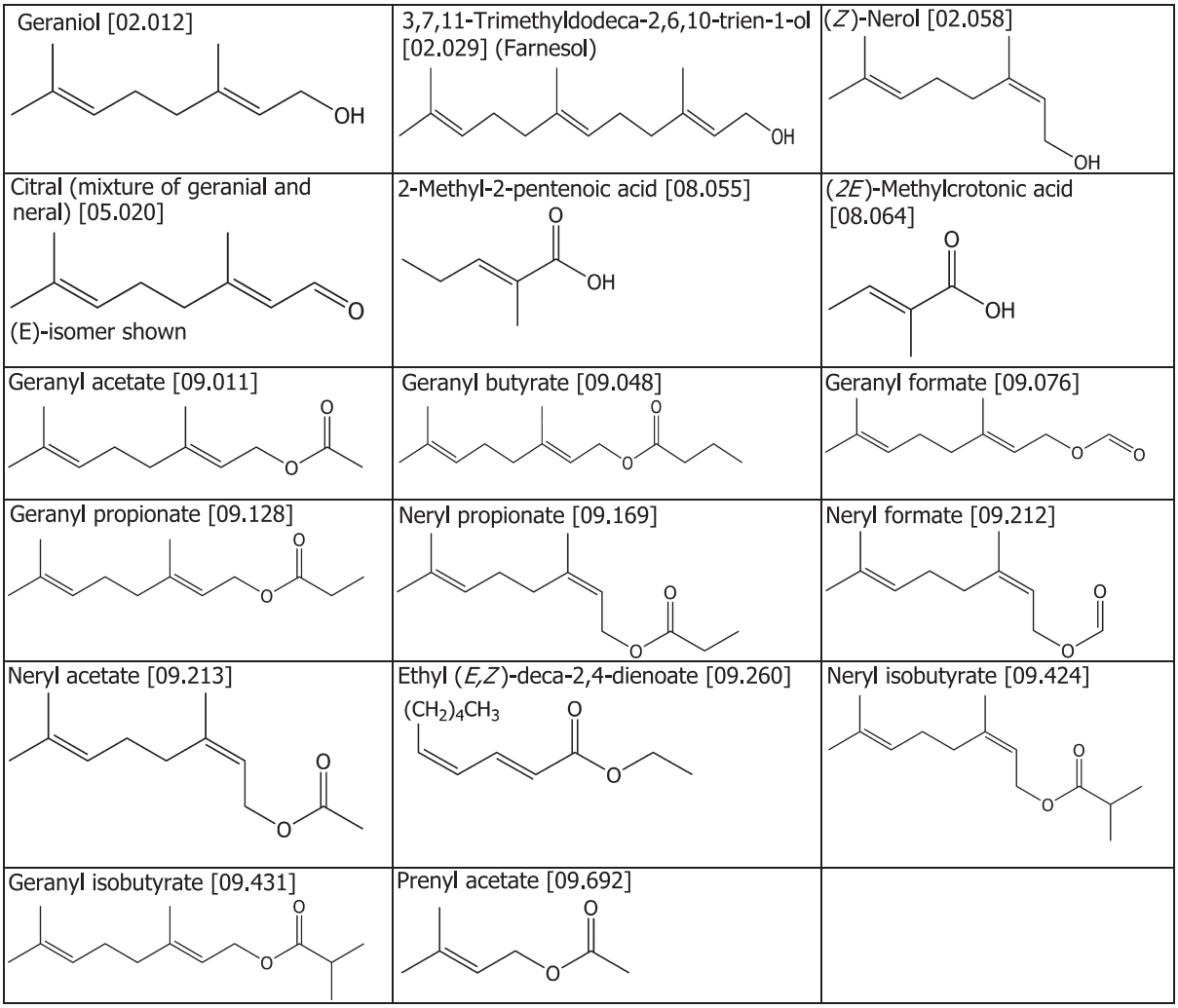

Figure 1: Molecular structures, [FLAVIS numbers] and (trivial names) of the 17 flavouring compounds under assessment

Table 1: Chemical Abstracts Service (CAS) and FLAVIS numbers and some characteristics of the chemically defined flavourings under assessment

EU register name	CAS no.	FLAVIS no.	Molecular formula	Molecular weight	Physical state	Log K_{ow} [(a),(b)]
Geraniol	106-24-1	02.012	$C_{10}H_{18}O$	154.25	Liquid	3.56
Farnesol	4602-84-0	02.029	$C_{15}H_{26}O$	222.37	Liquid	5.77
(*Z*)-Nerol	106-25-2	02.058	$C_{10}H_{18}O$	154.25	Liquid	3.47
Citral	5392-40-5	05.020	$C_{10}H_{16}O$	152.24	Liquid	3.45
2-Methyl-2-pentenoic acid	3142-72-1	08.055	$C_6H_{10}O_2$	114.14	Liquid	1.58
(*2E*)-Methylcrotonic acid	80-59-1	08.064	$C_5H_8O_2$	100.1	Liquid	1.40
Geranyl acetate	105-87-3	09.011	$C_{12}H_{20}O_2$	196.29	Liquid	4.04
Geranyl butyrate	106-29-6	09.048	$C_{14}H_{24}O_2$	224.34	Liquid	4.92
Geranyl formate	105-86-2	09.076	$C_{11}H_{18}O_2$	182.26	Liquid	3.93
Geranyl propionate	105-90-8	09.128	$C_{13}H_{22}O_2$	210.32	Liquid	4.41
Neryl propionate	105-91-9	09.169	$C_{13}H_{22}O_2$	210.31	Liquid	4.63
Neryl formate	2142-94-1	09.212	$C_{11}H_{18}O_2$	182.26	Liquid	3.93
Neryl acetate	141-12-8	09.213	$C_{12}H_{20}O_2$	196.29	Liquid	3.98
Ethyl (*E,Z*)-deca-2,4-dienoate	3025-30-7	09.260	$C_{12}H_{20}O_2$	196.29	Liquid	4.45
Neryl isobutyrate	2345-24-6	09.424	$C_{14}H_{24}O_2$	224.34	Liquid	5.38
Geranyl isobutyrate	2345-26-8	09.431	$C_{14}H_{24}O_2$	224.34	Liquid	5.38
Prenyl acetate	1191-16-8	09.692	$C_7H_{12}O_2$	128.17	Liquid	1.65

EU: European Union; Flavis number: EU Flavour Information System numbers.
(a): Logarithm of octanol–water partition coefficient.
(b): Taken from Pubchem or generated from EPI-Suite 4.01.

3,7,11-Trimethyldodeca-2,6,10-trien-1-ol [02.029] is hereafter referred as farnesol.

Geraniol [02.012] may be produced by fractional distillation of an essential oil or by chemical synthesis. The remaining compounds are all synthetically produced. Typical routes of synthesis are described for each compound.[9]

Data was provided on the batch to batch variation in five batches of each additive except neryl propionate [09.169] for which only one batch was provided owing to the low use volume (< 1 kg/year).[10] The content of the active substance exceeded the JECFA specifications for all compounds (Table 2), except neryl acetate [09.213] for which two batches were below 96%.

Table 2: Identity of the substances and data on purity

EU register name	FLAVIS no.	JECFA minimum specification %[(a)]	Assay %	
			Average	Range
Geraniol	02.012	88[(b)]	98.7	98.3–99.0
Farnesol	02.029	96[(c)]	98.0	96.9–99.3
(*Z*)-Nerol	02.058	95[(d)]	99.0	97.7–99.5
Citral[(e)]	05.020	96[(f)]	97.1	96.1–99.8
2-Methyl-2-pentenoic acid	08.055	98[(g)]	99.4	98.5–100
(*2E*)-Methylcrotonic acid	08.064	99[(h)]	99.6	99.1–99.9
Geranyl acetate	09.011	90[(i)]	98.8	96.3–99.8
Geranyl butyrate	09.048	92[(j)]	98.4	94.1–99.7
Geranyl formate	09.076	85[(k)]	96.7	95.2–97.8
Geranyl propionate	09.128	92[(l)]	98.1	95.4–99.7
Neryl propionate	09.169	95	98.2[(m)]	98.2
Neryl formate	09.212	90[(n)]	95.2	94.0–96.5

[9] Technical dossier/Section II.
[10] Technical dossier/Section II/Annex 2.1 and Supplementary information May 2011.

EU register name	FLAVIS no.	JECFA minimum specification %[a]	Assay %	
			Average	Range
Neryl acetate	09.213	96[o]	96.3	93.0–99.9
Ethyl (*E*,*Z*)-deca-2,4-dienoate	09.260	90[p,q]	99.2	98.2–99.6
Neryl isobutyrate	09.424	92[r]	99.1	98.0–99.8
Geranyl isobutyrate	09.431	95	97.4	96.8–98.5
Prenyl acetate	09.692	95[s]	99.2	98.6–99.7

EU: European Union; Flavis number: EU Flavour Information System numbers; JECFA: The Joint FAO/WHO Expert Committee on Food Additives.
(a): FAO (2006).
(b): The name geraniol specifies the (*E*)-isomer. According to JECFA: 'Min. Assay value is 88 (total alcohols as $C_{10}H_{18}O$) and secondary components citronellyl, neryl, and geranyl acetate esters' (EFSA CEF Panel, 2013a).
(c): Mixture of (*Z*)- and (*E*)-isomers for both C=C double bonds. 10–15% (2*Z*,6*Z*); 20–25% (2*E*,6*Z*); 20–25% (2*Z*,6*E*); 40–50% (2*E*,2*E*) (EFSA CEF Panel, 2013a).
(d): According to JECFA: 'Min. assay value is 95% (of total alcohols as $C_{10}H_{18}O$)' (EFSA CEF Panel, 2013a).
(e): JECFA evaluated the isomer mixture Citral (Mixture of geranial and neral), FLAVIS No [05.020]; CAS No 5392-40-5; JECFA No 1225. It contains unspecified amounts of neral [05.170].
(f): Mixture of (*Z*)- and (*E*)-isomer. CAS No in Register does not specify stereoisomeric composition (EFSA CEF Panel, 2013a).
(g): Mixture of (Z)- and (E)-isomer. CAS No in Register does not specify stereoisomeric composition. 60–75% (*E*) and 20–30% (*Z*) (EFSA CEF Panel, 2013a).
(h): Specification reflecting the use of the applicant members: minimum assay 98%.
(i): Secondary components: 4–6% geraniol and 1–2% nerol.
(j): Total esters. Secondary components: 3–5% geraniol and 1% nerol.
(k): Total esters. Secondary components: 8–10% geraniol and 2–4% nerol.
(l): Total esters. Secondary components: 3–4% geraniol and 1–2% nerol.
(m): One batch, use of the product 1 kg/year or less.
(n): Secondary components: 4–6% geraniol, 1–3% nerol and formate esters of citronellol, geraniol and rhodinol.
(o): Specification reflecting the use of the applicant members: minimum assay 93%.
(p): Secondary components: (*E*,*E*)-ethyl 2,4-decadienoate. Reflects the use of the applicant members. Specification indicates a minimum assay value of 98.0% (sum of isomers) with 92.0% (*E*,*Z*) and 0.1–8% of secondary isomers. Nevertheless, the minimum assay of JECFA is given.
(q): JECFA evaluated ethyl *trans*-2-*cis*-4-decadienate (CAS No as in the register). CAS No in the Register refers to the (2*E*,4*Z*)-enantiomer (EFSA, 2009b).
(r): Secondary components: 2–5% nerol and 1–2% geraniol.
(s): Specification reflecting the use of the applicant members: minimum assay 98%.

Potential contaminants are considered as part of the product specification and are monitored as part of the Hazard Analysis and Critical Control Point procedure applied by all consortium members. The parameters considered include residual solvents, heavy metals and other undesirable substances. However, no evidence of compliance was provided for these parameters.

3.1.2. Stability

The shelf life of the compounds under assessment ranges from 8 to 36 months, when stored in closed containers under recommended conditions. This assessment is made on the basis of compliance with the original specification over this storage period.

3.1.3. Conditions of use

The applicant proposes the use of all of the 17 compounds in feed for all animal species without withdrawal. For geraniol and citral, the applicant proposes a normal use level of 5 mg/kg feed and a high use level of 25 mg/kg. For the remaining 15 additives, the applicant proposes a normal use level of 1 mg/kg feed and a high use level of 5 mg/kg.

3.2. Safety

The assessment of safety is based on the high use levels proposed by the applicant (25 mg/kg for geraniol and citral, and 5 mg/kg for the remaining compounds).

3.2.1. Absorption, distribution, metabolism and excretion

In general, compounds belonging to CG 3 are rapidly absorbed, distributed, metabolised and excreted (WHO, 1999, EFSA CEF Panel, 2010b, 2013b).

The metabolic reactions involved in the biotransformation of straight-chain and branched-chain unsaturated primary aliphatic alcohols, aldehydes, carboxylic acids and esters (WHO, 1999; EFSA CEF Panel, 2010b, 2013b) are: (i) hydrolysis of esters (Heymann, 1980); (ii) oxidation of linear and branched-chain alcohols and aldehydes to acids, by high capacity nicotinamide adenine dinucleotide (NAD+)/(nicotinamide adenine dinucleotide phosphate) NADP-dependent enzymes (Voet and Voet, 1990; Feron et al., 1991; Parkinson, 1996); (iii) reduction of aldehydes to alcohols by NAD(P)H-dependent reductases; (iv) conjugation with glucuronic acid of alcohols (a minor pathway for primary alcohols) and polar metabolites resulting from a combination of ω,ω-1 and β-oxidation (Diliberto et al., 1990); (v) metabolism of the resulting linear- or branched-chain unsaturated carboxylic acids to carbon dioxide in the tricarboxylic acid cyclic and fatty acid pathway (Voet and Voet, 1990), by β-oxidation (linear- and short-chain branched carboxylic acids) or ω-oxidation (medium- and long-chain carboxylic acids, presence of ethyl or propyl side chains); (vi) isomerisation reactions by enoyl-CoA isomerase (shift of the double bond from delta^3- to delta^2-enoyl-CoA, when unsaturation begin at an odd-numbered carbon) and 3-hydroxyacyl-CoA epimerase (*cis*- to *trans*-isomerisation of delta^2-enoyl-CoA); and (vii) saturation of unsaturated short-chain acids, to yield a substrate that may participate in the fatty acid pathway.

Unsaturated carboxylic acids are metabolised by well recognised pathways leading to the production of acetyl-CoA and propionyl-CoA, which enter general metabolism (Stryer, 1988).

Metabolism studies in laboratory animals are available for geraniol [02.011], citral [05.020] and farnesol [02.029].

Male rats were given repeated oral doses of 800 mg [1-^{3}H]-geraniol/kg bw by gavage daily for 20 days. Two primary pathways leading to five urinary metabolites were identified. In one pathway, the alcohol is first oxidised to geranic acid (3,7-dimethyl-2,6-octadieneoic acid), which is subsequently hydrated to yield 3,7-dimethyl-3-hydroxy-6-octenoic acid. In a second pathway, the alcohol undergoes ω-oxidation mediated by liver cytochrome P-450 (Chadha and Madyastha, 1982) to yield 8-hydroxygeraniol. Selective oxidation at C8 yields 8-carboxygeraniol, which is further oxidised to the main urinary metabolite 2,6-dimethyl-2,6-octadienedioic acid (Chadha and Madyastha, 1984).

In rats, citral, a mixture of geranial (the corresponding aldehyde of geraniol) and its *cis*-isomer (neral), is metabolised via similar alcohol and ω-oxidation pathways. In male Fisher 344 rats given [1,2-^{14}C]citral at a dose of 5 or 500 mg/kg bw by gavage, citral was rapidly metabolised and excreted as metabolites. The major metabolite identified in the bile was the glucuronide of geranic acid. In the urine of these rats, several carboxylic acids were identified, among which the metabolites resulting from oxidation of the aldehyde function (geranic acid) or from ω-oxidation and further reduction and hydration of the double bond at C2 (Hildebrandt acid (2,6-dimethyl-2,6-octadienedioic acid) and dihydro-Hildebrandt acid) (Diliberto et al., 1990). Hepatic reduction of the aldehyde may precede oxidation pathways, as experiments *in vitro* revealed that citral is not oxidised by rat hepatic aldehyde dehydrogenase (ALDH) to the corresponding acids. In fact, citral was found to be a potent inhibitor of ALDH-mediated oxidation of acetaldehyde, and was reduced to the corresponding alcohols by rat hepatic alcohol dehydrogenase (ADH) (Boyer and Petersen, 1991). Nucleophilic addition reactions are common for α,β-unsaturated aldehydes but seem not to occur with citral as indicated by the absence of adducts with glutathione and other sulfhydryl compounds. Although citral has two carbon double bonds, which are potential sites for epoxidation, none of the metabolites observed in the urine of rats seem to arise from epoxides (Diliberto et al., 1990).

Farnesyldiphosphate (FPP) is an intermediate in the synthesis of cholesterol and subsequent steroids. Because of the biological role of farnesol, amounts of exogenous applied farnesol may also be incorporated into endogenous compounds, such as cholesterol. Farnesol plays an important role in the biochemistry of all eukaryotic cells. Although FPP is endogenously synthesised in cells of animals and humans, it has been shown that exogenously provided farnesol can also be phosphorylated by liver microsomes and peroxisomes (Westfall et al., 1997; Endo et al., 2011). Oxidative metabolism of farnesol by alcohol dehydrogenases of the liver and other organs (lung, colon, stomach) leads to the aldehyde farnesal, which can be either reduced back to farnesol by aldehyde reductase or further oxidised to farnesoic acid by microsomal aldehyde dehydrogenases. Excretion of farnesol is mediated by glucuronidation, which is mainly catalysed by UGT2B7 (Staines et al., 2004).

With the exception of a report describing the complete degradation/metabolism of nerol and geraniol after 24 h in a rumen simulation technique apparatus (Franz et al., 2010), studies on the metabolism of compounds belonging to CG 3 in target animals are lacking in the scientific literature. However, the enzymes involved in the biotransformation pathways of these compounds are present in all target species. Carboxylesterases, responsible for the hydrolysis of esters, are present in the gut especially of ruminants and liver of several animal species (cattle, pigs, broiler chicks, rabbits and horses), operating the hydrolysis of esters and originating the respective alcohols and acids (Gusson et al., 2006). Carboxylesterase activity also plays a significant role in detoxification processes in fish (Li and Fan, 1997; Di Giulio and Hinton, 2008). Reduction of aldehydes to alcohols can also been carried out by carbonyl reductases that are widely distributed in several animal species, including cattle, pig, rabbit, dog, sheep, and birds as reviewed by Felsted and Bachur (1980) and more recently evaluated *in vitro* in liver from cattle, pig, goat and sheep (Szotakova et al., 2004). β-Oxidation and ω-oxidation are endogenous pathways and are expected to occur in all animal species and β-oxidation has been demonstrated in fish (Crockett and Sidell, 1993) and birds (Pan and Fouts, 1978; Sanz et al., 2000). The CYP450 monooxygenase families, are present and have been characterised in a number of food-producing animals, including ruminants, horses, pigs, (Nebbia et al., 2003; Ioannides, 2006; Fink-Gremmels, 2008), fish (Wolf and Wolfe, 2005) and birds (Blevins et al., 2012). All these species, also carry out conjugation reactions with sulfate and glucuronic acid (Watkins and Klaassen, 1986; James, 1987; Gusson et al., 2006), producing water-soluble derivatives that are eliminated in urine. Therefore, mammals, fish and birds can be assumed to have the ability to metabolise and excrete the flavouring substances present in CG 3. The FEEDAP Panel notes that for feline species the capacity for conjugation is limited (Shrestha et al., 2011; Court, 2013).

3.2.2. Toxicological studies

Toxicological data (subchronic, repeated-dose studies, with multiple-doses tested) could be found only for geraniol [02.012], citral [05.020], geranyl acetate [09.011] and farnesol [02.0290].

A subchronic repeated-dose toxicity study (16 weeks, only one dose tested, by oral route, 5M/5F) in rat was available for geraniol [02.012] from a publication considering 48 flavourings (Hagan et al., 1967). The study considered a number of endpoints (survival, behaviour, body weight, feed intake; haematological parameters: white cell count, red cell count, haemoglobins and haematocrit; organ weight, gross pathology, histopathology). No effects were seen at the concentration tested (10,000 mg/kg diet corresponding to 500 mg/kg bw per day). In a further study reported in the same publication, no effects were seen when geraniol was administered with diet at 1,000 mg/kg (corresponding to 50 mg/kg bw per day) for 27–28 weeks. From the 90-day study, a no observed adverse effect level (NOAEL) of 10,000 mg/kg feed corresponding to 500 mg/kg bw per day could be derived for geraniol.

In the same publication (Hagan et al., 1967), a 13-week study in rats (males/females, 10 animals/sex and group) three doses of geranyl acetate (0, 1,000, 2,500 and 10,000 mg/kg equivalent to 0, 50, 125, 500 mg/kg bw per day) were administered via diet. No effects were seen on the same parameters described for geraniol at all the concentrations tested. From this study, also a NOAEL of 10,000 mg/kg feed corresponding to 500 mg/kg bw per day could be derived for geranyl acetate.

The subchronic toxicity of geranyl acetate was also tested in mice and rats dosed with a mixture containing also citronellyl acetate (test material: 71% geranyl acetate, 29% citronellyl acetate) (NTP, 1987). The mixture was administered by gavage at doses of 0, 125, 500 and 1,000 mg/kg bw per day in mice (males/females, 10 animals/sex and group) and of 0, 250, 500, 1,000, 2,000 and 4,000 mg/kg bw per day in rat (males/females, 10 animals/sex and group). No adverse effects were seen. The same mixture was not considered carcinogenic to mice and rats in a 2-year study, when administered at doses of 500 and 1,000 mg/kg bw in mice and of 1,000 and 2,000 mg/kg bw in rat (NTP, 1987).

Several studies were available for citral [05.020] and were reviewed by the EFSA CEF Panel (2013b). No treatment-related effects on growth, haematology and organ weights were observed, and there were no macroscopic or microscopic changes in tissues when citral was administered to rats at doses of 0, 50, 125 and 500 mg/kg bw per day for 13 weeks (Hagan et al., 1967). This study also confirms a NOAEL of 500 mg/kg bw per day.

In a 14-week study (NTP, 2003), higher doses of citral were given via feed to mice (0, 745, 1,840, 3,915 and 8,810 mg/kg bw per day in males and 0, 790, 1,820, 3,870 and 7,550 mg/kg bw per day in females) and rat (0, 345, 820, 1,785 mg/kg bw per day in males and 0, 335, 675, and 1,330 mg/kg bw per day in females). In mice, decreased body weights and body-weight gains, and a reduction in

the lymphocyte count in males, and decreased body weights and body-weight gains in females were observed at all dose levels, indicating that the NOAEL is < 745 and < 790 mg/kg bw per day in males and females, respectively. In rats, significantly increased incidences of nephropathy and granular casts (characterised by dilated tubules) were seen in males, at the two highest doses. Decreased body weights and body-weight gains, and bone marrow atrophy accompanied by bone marrow haemorrhage in females were observed at the highest dose. The NOAEL derived from this study is 675 mg/kg bw per day in females and 345 mg/kg bw per day in males.

A 2-year carcinogenicity study was performed with rats and mice (50 males and females each) fed diets containing 1,000, 2,000 or 4,000 mg/kg feed microencapsulated citral (corresponding to approximately 50, 100 and 210 mg/kg bw in rat and 60, 120 and 260 mg/kg bw in mice). Additional groups of 50 male and 50 female rats received untreated feed (untreated controls) or feed containing placebo microcapsules (vehicle controls) (NTP, 2003). The only treatment-related effects observed after 2 years were weight reductions at 210 mg/kg bw in rats and 120 mg/kg bw in mice. Although the body weight effect was selected by JECFA as the NOAEL for calculating an ADI, the differences are rather small and it is unclear whether they are statistically significant. Although the feed intakes of the groups are similar the possibility that this is a palatability effect cannot be excluded, as this appeared to be the primary reason for body weight differences seen in the 90-day study. It is considered by the Panel that the body weight differences in the 2-year studies do not represent an adverse effect, particularly as the survival of those groups was in fact enhanced. The conclusion is therefore that for rats and mice the 2-year NOAEL is > 210 mg/kg bw per day.

The FEEDAP Panel selects a NOAEL of 345 mg/kg bw per day from the 90-day study in rat (NTP, 2003) as a group NOAEL for citral, geraniol, (*Z*)-nerol (the *cis*-isomer of geraniol) and related esters.

The toxicity of subchronic oral administration of farnesol and the influence of the test material on the activity of hepatic and renal drug-metabolising enzymes was studied in CD rats. Administration of farnesol (composed of four isomers: *cis,cis*- (11.1%), *cis,trans*- (25.1%), *trans,cis*- (24.6%) and *trans,trans*- (38.8%)) at 500 or 1,000 mg/kg per day in corn oil for 28 days induced dose-related alterations in liver and kidney weights and elevated the drug-metabolising enzyme activities of the liver (CYP2E1, glutathione reductase and NADPH quinone reductase) and kidney (glutathione-*S*-transferase), but all effects were reversible. No effects on body weight, food consumption, clinical signs and haematology parameters were observed. Enzyme activities did not differ from control after the 28-day recovery period. Hence, the NOAEL was determined to be 1,000 mg/kg bw per day (Horn et al., 2004, 2005).

A publication by Lindecrona et al. (2003) investigated the subchronic oral toxicity of octan-3-ol, 2-methylcrotonic acid and the resulting ester oct-3-yl 2-methylcrotonate. Octan-3-ol was administered by gavage to rats (10 females and 10 males) at three dose levels (0, 25, 100 or 400 mg/kg bw per day) with the aim of establishing a NOAEL for this substance. For the purpose of comparing the relative toxicity of the ester and its components, the carboxylic acid and the alcohol, only one dose was tested for 2-methylcrotonic acid (77 mg/kg bw per day) and oct-3-yl 2-methylcrotonate (163 mg/kg bw per day). The design of the study does not allow establishing a NOAEL for 2-methylcrotonic acid [08.064].

3.2.3. Safety for the target species

The first approach to the safety assessment for target species takes account of the intended use levels in animal feed relative to the maximum reported exposure of humans on the basis of the metabolic body weight. Human exposure in the EU to the individual compounds ranges from 0.0061 to 5,844 μg/person per day (EFSA, 2009b; EFSA CEF Panel, 2010a, 2013b). This corresponds to 0.0003–271 $\mu g/kg^{0.75}$ per day. These exposure levels are considered safe for humans. Table 3 summarises the result of the comparison with human exposure for representative target animals.

Table 3: Comparison of exposure of humans and target animals (calculated from the proposed maximum feed concentrations of 5 or 25 mg/kg feed) to the flavourings under application

Flavouring	Use level in feed (mg/kg)	Human exposure ($\mu g/kg\ bw^{0.75}$ per day)[(a)]	Target animal exposure $\mu g/kg\ bw^{0.75}$ per day		
			Salmon	Piglet	Dairy cow
Geraniol (*Z*-isomer)	25	25.5	588	2,632	3,885
Farnesol	5	0.36	118	526	777
(*Z*)-Nerol	5	11.6	118	526	777

Flavouring	Use level in feed (mg/kg)	Human exposure (μg/kg bw$^{0.75}$ per day)[a]	Target animal exposure μg/kg bw$^{0.75}$ per day		
			Salmon	Piglet	Dairy cow
Citral	25	271	588	2,632	3,885
2-Methyl-2-pentenoic acid	5	1.67	118	526	777
(*2E*)-Methylcrotonic acid	5	0.009	118	526	777
Geranyl acetate	5	21.8	118	526	777
Geranyl butyrate	5	2.41	118	526	777
Geranyl formate	5	13.0	118	526	777
Geranyl propionate	5	3.20	118	526	777
Neryl propionate	5	0.17	118	526	777
Neryl formate	5	0.0003	118	526	777
Neryl acetate	5	6.96	118	526	777
Ethyl (*E,Z*)-deca-2,4-dienoate	5	1.35	118	526	777
Neryl isobutyrate	5	0.08	118	526	777
Geranyl isobutyrate	5	5.10	118	526	777
Prenyl acetate	5	0.93	118	526	777

(a): Metabolic body weight (kg bw$^{0.75}$) for a 60-kg person = 21.6.

Table 3 shows that for all compounds the intake by the target animals greatly exceeds that of humans, resulting from use in food.

Safety for the target species at the feed concentration applied cannot be derived from the risk assessment for food use. As an alternative, the maximum feed concentration which can be considered safe for the target animals can be derived from the lowest NOAEL if suitable data are available.

Toxicological data derived from a subchronic, repeated-dose study were available for geraniol [02.012], citral [05.020], geranyl acetate [09.011] and farnesol [02.029] (see Section 3.2.2). A group NOAEL of 345 mg/kg bw per day is considered to apply to geraniol [02.012], citral [05.020], geranyl acetate [09.011], geranyl butyrate [09.048], geranyl formate [09.076], geranyl proprionate [09.128], geranyl isobutyrate [09.431], (*Z*)-nerol [02.058] (the *cis*-isomer of geraniol) and its esters neryl propionate [09.169], neryl formate [09.212] and neryl acetate [09.213]. For farnesol [02.029], a NOAEL of 1,000 mg/kg bw per day was derived from a 28-day toxicity study in rat. Applying an uncertainty factor (UF) of 100 to the NOAELs derived from chronic and subchronic studies and an additional UF of 2 to the NOAEL derived from a 28-day study with farnesol, the maximum safe intake for the target species was derived following the EFSA Guidance for sensory additives (EFSA FEEDAP Panel, 2012a), and thus the maximum safe feed concentration was calculated (Table 4).

Table 4: Maximum safe concentration in feed for different target animals for (**A**) citral, geraniol, (*Z*)-nerol and related esters (NOAEL (345 mg/kg bw per day); (**B**) farnesol (NOAEL 1,000 mg/kg bw with an UF of 200)

Target animal	Default values		Maximum safe intake/feed concentration			
	Body weight (kg)	Feed intake (g/day)[a]	Intake (mg/day)		Concentration (mg/kg feed)[b]	
			A	B	A	B
Salmonids	2	40	7	10	173	251
Veal calves (milk replacer)	100	2,000	345	500	173	250
Cattle for fattening	400	8,000	1,380	2,000	152	220
Dairy Cows	650	20,000	2,243	3,250	99	143
Piglets	20	1,000	69	100	69	100
Pigs for fattening	100	3,000	345	500	115	167
Sows	200	6,000	690	1,000	115	167
Chickens for fattening	2	120	7	10	57	83

Target animal	Default values		Maximum safe intake/feed concentration			
	Body weight (kg)	Feed intake (g/day)[a]	Intake (mg/day)		Concentration (mg/kg feed)[b]	
			A	B	A	B
Laying hens	2	120	7	10	57	83
Turkeys for fattening	12	400	41	60	104	150
Dogs	15	250	52	75	182	264
Cats[c]	3	60	2	3	30	44

(a): Complete feed with 88% dry matter (DM), except milk replacer for veal calves (94.5% DM), and for cattle for fattening, dairy cows, dogs and cats for which the values are DM intake.
(b): Complete feed containing 88% DM, milk replacer 94.5% DM.
(c): The uncertainty factor (UF) for cats is increased by an additional factor of 5 because of the reduced capacity for glucuronidation.

As individual reliable NOAELs could not be found for the remaining four compounds, the threshold of toxicological concern (TTC) approach was followed to derive the maximum safe feed concentration (EFSA FEEDAP Panel, 2012a).

For Cramer class I compounds, i.e. 2-methyl-2-pentenoic acid [08.055], (2*E*)-methylcrotonic acid [08.064], ethyl (*E,Z*)-deca-2,4-dienoate [09.260] and prenyl acetate [09.692], the calculated safe use level is 1.5 mg/kg complete feed for cattle, salmonids and non-food-producing animals and 1.0 mg/kg complete feed for pigs and poultry.

3.2.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that:

- geraniol [02.012] and citral [05.020] are safe at the proposed maximum use level of 25 mg/kg feed for all target species;
- farnesol [02.029], (*Z*)-nerol [02.058], geranyl acetate [09.011], geranyl butyrate [09.048], geranyl formate [09.076], geranyl propionate [09.128], neryl propionate [09.169], neryl formate [09.212], neryl acetate [09.213], neryl isobutyrate [09.424] and geranyl isobutyrate [09.431] are safe at the proposed maximum use level of 5 mg/kg feed for all target species;
- 2-methyl-2-pentenoic acid [08.055], (*2E*)-methylcrotonic acid [08.064], ethyl (*E,Z*)-deca-2,4-dienoate [09.260] and prenyl acetate [09.692] are safe at the proposed normal use levels of 1 mg/kg complete feed for all animal species.

3.2.4. Safety for the consumer

The safety for the consumer of the 17 compounds used as food flavours has been already assessed by JECFA (WHO, 1999, 2004, 2005) and EFSA (EFSA, 2008, EFSA CEF Panel, 2013a,b). All compounds are currently authorised in the EU as food flavourings without limitations.[11]

Deposition and residue studies of the compounds in farm animals are not available. However, there is limited data which indicates that grazing animals are naturally exposed to some of the compounds of CG 3 and these can be found in dairy products. Geranyl acetate was identified in cheese made with cow milk from pasture but not with milk from total mixed rations (Carpino et al., 2004). Also, nerol is reported to be in significantly higher quantities in cheese made with cow milk from highland grazing as compared with lowland pasture (Bosset et al., 1994). Geraniol was identified in cheese produced with milk from natural grassland grazed cows (Cornu et al., 2005).

Given the low use levels of CG 3 compounds to be applied in feed, and the expected extensive metabolism and excretion in target animals (see Section 3.2.1), the FEEDAP Panel considers that the use of these flavourings in animal feed would not appreciably increase the human exposure to these compounds.

[11] 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.

3.2.5. Safety for the user

No specific data on the safety for the user were provided. In the material safety data sheets,[12] hazards for skin and eye contact and respiratory exposure are recognised for the majority of the compounds under application. Most are classified as irritating to the respiratory system. Some of the compounds (e.g. geraniol, citral and farnesol) are also reported to be skin sensitisers, however, the sensitising potential is most probably caused by peroxides formed during contact of the unsaturated terpenes with air rather than the pure compounds (Hagvall et al., 2007).

3.2.6. Safety for the environment

The additions of naturally occurring substances that will not result in a substantial increase in the concentration in the environment are exempt from further assessment. Examination of the published literature shows that this applies to 12 substances, namely geraniol [02.012], 3,7,11-trimethyldodeca-2,6,10-trien-1-ol [02.029], (*Z*)-nerol [02.058], citral (mixture of geranial and neral) [05.020], geranyl acetate [09.011], geranyl butyrate [09.048], geranyl formate [09.076], geranyl propionate [09.128], neryl propionate [09.169], neryl acetate [09.213], ethyl (*E,Z*)-deca-2,4-dienoate [09.260] and geranyl isobutyrate [09.431], which occur in the environment at levels above the application rate of 5 mg/kg feed. (Data taken from the Netherlands Organisation for Applied Scientific Research (TNO) database Volatile Compounds in Food *ver.* 14.1; Burdock, 2009).[13]

The other five compounds, namely 2-methyl-2-pentenoic acid [08.055], (*2E*)-methylcrotonic acid [08.064], neryl formate [09.212], neryl isobutyrate [09.424] and prenyl acetate [09.692], could not be shown to occur in the environment at levels above the application rate of 1–5 mg/kg feed.

However, the FEEDAP Panel considers that there is a high probability of complete hydrolysis of the three esters in the target animal, resulting in (*Z*)-nerol and prenol which are naturally occurring compounds. Therefore, neryl formate [09.212], neryl isobutyrate [09.424] and prenyl acetate [09.692] are excluded from further assessment.

For the remaining two compounds, 2-methyl-2-pentenoic acid [08.055] and (2*E*)-methylcrotonic acid [08.064], the predicted environmental concentration for soil (PEC_{soil}) was calculated based on the use rate (Table 5) and compared with the trigger values for compartments set in the Phase I of EFSA guidance (EFSA, 2008).

Table 5: Predicted environmental concentration (PEC) values of 2-methyl-2-pentenoic acid [08.055] and (*2E*)-methylcrotonic acid [08.064]

EU register name	CAS no.	Dose (mg/kg)	PEC_{soil} (µg/kg)	$PEC_{pore\ water}$ (µg/L)	$PEC_{surface\ water}$ (µg/L)
2-Methyl-2-pentenoic acid	3142-72-1	1.5	32	125	42
2-Methylcrotonic acid	80-59-1	1.5	32	165	55

EU: European Union; PEC: predicted environmental concentration; CAS no.: Chemical Abstracts Service number.

PEC_{soil} are above the threshold of 10 µg/kg (EFSA, 2008). The PEC for pore water, however, is dependent on the sorption, which is different for each compound. For these calculations, the substance-dependent constants organic carbon sorption constant (K_{oc}), molecular weight, vapour pressure and solubility are needed. These were estimated from the Simplified Molecular Input Line Entry Specification (SMILES) notation of the chemical structure using EPIWEB 4.1 (Table 6).[14] This program was also used to derive the SMILES notation from the CAS numbers. The K_{oc} value derived from the first-order molecular connectivity index was used, as recommended by the EPIWEB program.

[12] Technical dossier/Section II/Annex II.3.
[13] Technical dossier/Supplementary information June 2011.
[14] Available online: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm

Table 6: Physicochemical properties predicted by EPIWEB 4.1 for 2-methyl-2-pentenoic acid [08.055] and (*2E*)-methylcrotonic acid [08.064]

EU Register name	CAS no.	Predicted by EPIWEB 4.1				
		DT_{50}[(a)] (days)	Molecular weight (g/mol)	Vapour pressure (Pa)	Solubility (mg/L)	K_{oc}[(b)] (L/kg)
2-Methyl-2-pentenoic acid	3142-72-1	4	114.15	23.7	6,330	8
(*2E*)-Methylcrotonic acid	80-59-1	3	100.12	59.7	18,450	4

EU: European Union; CAS no.: Chemical Abstracts Service number.
(a): DT_{50}, half-life of the additive (by BioWin 3).
(b): K_{oc}, organic carbon sorption constant.

The half-life (DT_{50}) was calculated using BioWin3 (Ultimate Survey Model), which gives a rating number. This rating number r was translated into a half-life using the formula by Arnot et al. (2005):

$$DT_{50} = 10^{(-r \times 1.07 + 4.12)}$$

This is the general regression used to derive estimates of aerobic environmental biodegradation half-lives from BioWin3 model output.

Both substances in Table 5 have $PEC_{pore\ water}$ above 0.1 μg/L and a PEC_{soil} above 10 μg/kg. Therefore, these two substances are subjected to phase II risk assessment.

In the absence of experimental data, the phase II risk assessment was performed using ECOSAR v1.11, which estimates the half-maximal effective concentration (EC_{50}) or lethal concentration (LC_{50}) for earthworms, fish, algae and daphnids from the SMILES notation of the substance. The predicted no effect concentration (PNEC) for terrestrial environment ($PNEC_{soil}$) was determined by dividing the LC_{50} earthworm by a UF of 1,000. The corresponding PNEC for aquatic compartment ($PNEC_{aquatic}$) was derived from the lowest toxicity value for freshwater environment by applying an UF of 1,000.

For both compounds, the ratio PEC/PNEC for soil and surface water was < 1, indicating that there is no risk for the terrestrial and freshwater environment at the use levels considered safe for target species (Table 7).

Table 7: Phase II environmental risk assessment of soil and aquatic compartments for 2-methyl-2-pentenoic acid [08.055] and (*2E*)-methylcrotonic acid [08.064] when used as feed additives for terrestrial farm animals (exposure and effect data were modelled using EPIWEB 4.1 and ECOSAR 1.11)

EU register name Soil	LC_{50}[(a)] earthworm (mg/kg)			$PNEC_{soil}$ (μg/kg)	PEC_{soil} (μg/kg)	PEC/PNEC
2-Methyl-2-pentenoic acid	2,036			2,036	32	0.02
(*2E*)-Methylcrotonic acid	2,008			2,008	32	0.02
Aquatic	LC_{50} Fish (mg/L)	LC_{50} Daphnids (mg/L)	EC_{50}[(b)] Algae (mg/L)	$PNEC_{aquatic}$ (μg/L)	PEC_{sw}[(c)] (μg/L)	PEC/PNEC
2-Methyl-2-pentenoic acid	1,171	659	471	471	42	0.1
(*2E*)-Methylcrotonic acid	2,837	1,524	903	903	55	0.1

EU: European Union; PNEC: predicted no effect concentration.
(a): LC_{50}: the concentration of a test substance which results in a 50% mortality of the test species.
(b): EC_{50}: the concentration of a test substance which results in 50% of the test animals being adversely affected (i.e. both mortality and sublethal effects).
(c): PEC_{sw}: predicted environmental concentration in surface water.

The use of all additives in fish feed in land-based aquaculture systems does not give a PEC of the additive (parent compound) in surface water (PEC_{swaq}) above the trigger value of 0.1 μg/L when calculated according to the guidance. For sea cages, a dietary concentration of 0.047 mg/kg would ensure that the threshold for the PEC of the additive (parent compound) in sediment (PEC_{sed}) of 10 μg/kg is not exceeded when calculated according to the EFSA guidance (EFSA, 2008).

3.2.6.1. Conclusions on safety for the environment

The concentrations considered safe for the target species (see Section 3.1.1) are unlikely to have detrimental effects on the terrestrial and fresh water environments. For the marine environment, the safe use level is estimated to be 0.05 mg/kg feed.

3.3. Efficacy

As the 17 compounds are used in food as flavourings, and their function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

4. Conclusions

The FEEDAP Panel concludes that geraniol [02.012] and citral [05.020] are safe for all target species at the proposed maximum use level of 25 mg/kg feed; farnesol [02.029], (*Z*)-nerol [02.058], geranyl acetate [09.011], geranyl butyrate [09.048], geranyl formate [09.076], geranyl propionate [09.128], neryl propionate [09.169], neryl formate [09.212], neryl acetate [09.213], neryl isobutyrate [09.424] and geranyl isobutyrate [09.431] are safe at the maximum proposed use level of 5 mg/kg feed for all target species; 2-methyl-2-pentenoic acid [08.055], (*2E*)-methylcrotonic acid 08.064], ethyl (*E,Z*)-deca-2,4-dienoate [09.260] and prenyl acetate [09.692] are safe at the proposed normal use levels of 1 mg/kg complete feed for all animal species.

No safety concern would arise for the consumer from the use of these compounds up to the highest safe levels in feed.

Hazards for skin and eye contact and respiratory exposure are recognised for the majority of the compounds under application. Most are classified as irritating to the respiratory system.

The concentrations considered safe for the target species are unlikely to have detrimental effects on the terrestrial and fresh water environments.

As all of the compounds under assessment are used in food as flavourings and their function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

Documentation provided to EFSA

1) Chemically Defined Group 03 - α,β-unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters with esters containing α,β-unsaturated alcohols or aldehydes. September 2010. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
2) Chemically Defined Group 03 - α,β-unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters with esters containing α,β-unsaturated alcohols or aldehydes. July 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
3) Chemically Defined Group 03 - α,β-unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters with esters containing α,β-unsaturated alcohols or aldehydes. July 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Chemically Defined Group 03 - α,β-unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters with esters containing α,β-unsaturated alcohols or aldehydes.
5) Comments from Member States.

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Abbreviations

ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CDG	chemically defined group
DM	dry matter
DT_{50}	degradation half-time
EC_{50}	half-maximal effective concentration
ECOSAR	component program of EPI suite™
EPI suite	Estimation Programs Interface (EPI) Suite™
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed

FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	EU Flavour Information System
FPP	farnesyldiphosphate
GC–MS	gas chromatography–mass spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K_{oc}	organic carbon sorption constant
K_{ow}	octanol–water partition coefficient
LC_{50}	lethal concentration 50
Log K_{ow}	logarithm of octanol–water partition coefficient
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NOAEL	no observed adverse effect level
PEC	predicted environmental concentration
$PEC_{pore\ water}$	predicted environmental concentration for pore water
PEC_{sed}	predicted environmental concentration of the additive (parent compound) in sediment
PEC_{soil}	predicted environmental concentration for soil
$PEC_{surface\ water}$	predicted environmental concentration for surface water
PEC_{swaq}	predicted environmental concentration of the additive (parent compound) in surface water
PNEC	predicted no effect concentration
$PNEC_{soil}$	predicted no effect concentration for terrestrial environment
$PNEC_{aquatic}$	predicted no effect concentration of aquatic compartment
RTL	retention time locking
SMILES	Simplified Molecular Input Line Entry Specification
TNO	Netherlands Organisation for Applied Scientific Research
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Chemically Defined Flavourings - Group 03 α,β-unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters with esters containing α,β-unsaturated alcohols or aldehydes

The *Chemically Defined Flavourings - Group 03 (α,β-unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters with esters containing α,β-unsaturated alcohols or aldehydes)*, in this application comprises 43 substances, for which authorisation as feed additives is sought under the category 'sensory additives', functional group 2(b) 'flavouring compounds', according to the classification system of Annex I of Regulation (EC) No 1831/2003.

In the current application submitted according to Article 4(1) and Article 10(2) of Regulation (EC) No 1831/2003, the authorisation for all species and categories is requested. The flavouring compounds of interest have a purity ranging from 85% to 98%.

Mixtures of flavouring compounds are intended to be incorporated only into *feedingstuffs* or drinking *water*. The Applicant suggested no minimum or maximum levels for the different flavouring compounds in *feedingstuffs*.

For the identification of volatile chemically defined flavouring compounds *CDG03* in the *feed additive*, the Applicant submitted a qualitative multianalyte gas chromatography–mass spectrometry (GC–MS) method, using retention time locking (RTL), which allows a close match of retention times on GC–MS. By making an adjustment to the inlet pressure, the retention times can be closely matched to those of a reference chromatogram. It is then possible to screen samples for the presence of target compounds using a mass spectral database of RTL spectra. The Applicant maintained two FLAVOR2 databases/libraries (for retention times and for MS spectra) containing data for more than 409 flavouring compounds. These libraries were provided to the European Union Reference Laboratory (EURL). The Applicant provided the typical chromatogram for the *CDG03* of interest.

In order to demonstrate the transferability of the proposed analytical method (relevant for the method verification), the Applicant prepared a model mixture of flavouring compounds on a solid carrier to be identified by two independent expert laboratories. This mixture contained twenty chemically defined flavourings belonging to twenty different chemical groups to represent the whole spectrum of compounds in use as feed flavourings with respect to their volatility and polarity. Both laboratories properly identified all the flavouring compounds in all the formulations. Since the substances of *CDG03* are within the volatility and polarity range of the model mixture tested, the Applicant concluded that the proposed analytical method is suitable to determine qualitatively the presence of the substances from *CDG03* in the mixture of *flavouring compounds*.

Based on the satisfactory experimental evidence provided, the EURL recommends for official control for the qualitative identification in the *feed additive* of the individual (or mixture of) flavouring compounds of interest the GC–MS–RTL (Agilent specific) method submitted by the Applicant. However, the method is not able to discriminate between [nona-2,6-dien-1-ol and tr-2, *cis*-6-nonadien-1-ol] or [2-dodecenal and dodec-2(*trans*)-enal] or [nona-2,4-dienal and tr-2,tr-4-nonadienal] or [2,4-decadienal and deca-2(*trans*),4(*trans*)-dienal] or [*trans*-2-nonenal and non-2-enal].

As no experimental data were provided by the Applicant for the identification of the *active substance(s)* in *feedingstuffs* and *water*, no methods could be evaluated. Therefore, the EURL is unable to recommend a method for the official control to identify the *active substance*(s) of interest in *feedingstuffs* or *water*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.