SCIENTIFIC OPINION

ADOPTED: 1 December 2015 PUBLISHED: 05 January 2016

doi:10.2903/j.efsa.2016.4339

Safety and efficacy of eight compounds belonging to chemical group 31 (aliphatic and aromatic hydrocarbons) when used as flavourings for all animal species and categories

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 Panel) was asked to deliver a scientific opinion on the safety and efficacy of 17 compounds belonging to chemical group 31 (aliphatic and aromatic hydrocarbons). They are currently authorised for use as flavours in food. This opinion concerns eight compounds from this group. The FEEDAP Panel concluded that β-pinene, α-pinene, β-caryophyllene, myrcene, camphene, β-ocimene and δ-3-carene are safe at the proposed maximum dose level (5 mg/kg complete feed) for all animal species, except myrcene and β-ocimene when 4 mg/kg would apply for cats. For valencene, 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. No safety concern would arise for the consumer from the use of these compounds up to the highest safe levels in feeds. The Panel is unable to conclude on user safety in the absence of specific data. The use of the compounds in animal nutrition would not pose a risk for the environment provided that the concentrations regarded as safe for the target species are not exceeded. 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. However, in the absence of data on the stability/survival in water for drinking, the FEEDAP Panel is unable to conclude on the safety or efficacy of the substances under this mode of delivery.

Keywords: sensory additives, aliphatic and aromatic hydrocarbons, chemical group 31, safety

Requestor: European Commission

Question number: EFSA-Q-2015-00069

Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Feed Flavourings, including Paul Brantom, Birgit Dusemund, Christer Hogstrand, Anne-Katrine Lundebye, Robert Luttik, Derek Renshaw, Patrick Van Beelen and Johannes Westendorf, for the support provided to this scientific output.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of aliphatic and aromatic hydrocarbons (chemical Group 31) when used as flavourings for all animal species and categories. EFSA Journal 2016; 14(1):4339, 17 pp. doi:10.2903/j.efsa.2016.4339

ISSN: 1831-4732

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

Table of contents

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 seven years after the entry into force of this Regulation.

The European Commission (EC) received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation of 17 substances belonging to chemical group 31,[3] when used as a feed additive for all animal species (category: sensory additives; functional group: flavourings). Chemical group (CG) 31 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[4] as "aliphatic and aromatic hydrocarbons".

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 9 June 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 the pin-2(10)-ene, pin-2(3)-ene, β-caryophyllene, myrcene, camphene, valencene, β-ocimene and δ-3-carene, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The initial application concerned 17 compounds assigned to this CG, intended to be used as feed flavourings for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has already delivered an opinion on nine of the 17 compounds (EFSA FEEDAP Panel, 2015).

The remaining eight compounds were excluded from the previous assessment because the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) had requested additional genotoxicity and toxicity data to complete their assessment. The EFSA CEF Panel has now delivered an opinion on the eight compounds, concluding that there are no genotoxicity concerns (EFSA CEF Panel, 2015a).

Consequently, this opinion deals with the eight compounds, namely pin-2(10)-ene (hereafter referred to as β-pinene) [01.003], pin-2(3)-ene (hereafter referred to as α-pinene) [01.004], β-caryophyllene [01.007], myrcene [01.008], camphene [01.009], valencene [01.017], β-ocimene [01.018] and δ-3-carene [01.029], excluded from the previous opinion.

[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 FFAC EEIG was liquidated on 19/12/2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures). Avenue Louise 130A, Box 1, 1050 Brussels, Belgium.

[3] During the course of the assessment, this application was split and the present opinion covers only 8 out of the 17 substances under application (see 1.2).

[4] 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.

The eight compounds are currently listed in the European Union database of flavouring substances[5] and in the European Union Register of Feed Additives, respectively, 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 aliphatic and aromatic hydrocarbons as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003 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 aliphatic and aromatic hydrocarbons in animal feed. The Executive Summary of the EURL report can be found in Annex A.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the aliphatic and aromatic hydrocarbons is consistent 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, revised in 2009), 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 additives

The molecular structures of the eight additives under application are shown in Figure 1 and their physico-chemical characteristics in Table 1. Six compounds are monoterpenes (four bicyclic and two acyclic) and the remaining two (β-caryophyllene and valencene) are sesquiterpenes.

[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-2010-0411.

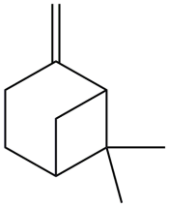
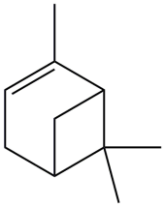
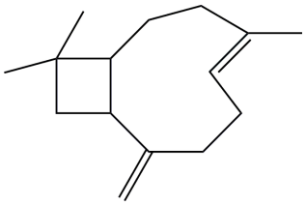
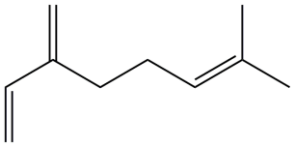
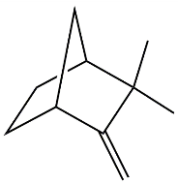
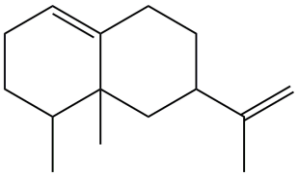
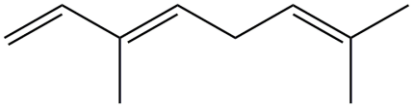
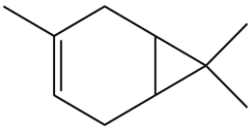
β-Pinene (Pin-2(10)-ene) [01.003] 	α-Pinene (Pin-2(3)-ene) [01.004] 	β-Caryophyllene [01.007] 
Myrcene [01.008] 	Camphene [01.009] 	Valencene [01.017] 
β-Ocimene [01.018] 	δ-3-Carene [01.029] 	

Figure 1: Molecular structures and FLAVIS numbers of the eight flavouring compounds under assessment

Table 1: Chemical Abstract Services (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} (1)
Pin-2(10)-ene (β-pinene)	127-91-3	01.003	$C_{10}H_{16}$	136.24	Liquid	4.16
Pin-2(3)-ene (α-pinene)	80-56-8	01.004	$C_{10}H_{16}$	136.24	Liquid	4.83
β-Caryophyllene	87-44-5	01.007	$C_{15}H_{24}$	204.36	Liquid	6.3
Myrcene	123-35-3	01.008	$C_{10}H_{16}$	136.24	Liquid	4.17
Camphene	79-92-5	01.009	$C_{10}H_{16}$	136.24	Solid	4.35
Valencene	4630-07-3	01.017	$C_{15}H_{24}$	204.36	Liquid	-
β-Ocimene	13877-91-3	01.018	$C_{10}H_{16}$	136.24	Liquid	4.8
δ-3-Carene	13466-78-9	01.029	$C_{10}H_{16}$	136.24	Liquid	4.38

(1): Logarithm of octanol–water partition coefficient.

These substances are produced either by chemical synthesis or by extraction from natural sources. Several routes of synthesis are available and described in the dossier for all compounds.[8]

Batch-to-batch variation data were provided for five batches of each additive, except for α-pinene [01.004] for which the analysis of four batches was available. The content of the active substance for all compounds exceeded the JECFA specifications (Table 2).

[8] Technical dossier/Section II.

Table 2: Identity of the substances and data on purity

EU Register name	Flavis no	JECFA specification minimum %	Assay %	
			Average	Range
Pin-2(10)-ene (β-pinene)	01.003	97	99.1	98.5–99.6
Pin-2(3)-ene (α-pinene)	01.004	98	99.0	97.6–99.5
β-Caryophyllene	01.007	80	96.6	90.0–97.2
Myrcene	01.008	90	93.9	91.2–96.1
Camphene	01.009	80	95.0	90.5–98.0
Valencene	01.017	94	96.0	94.4–97.1
β-Ocimene	01.018	80	97.0	88.7–99.4
δ-3-Carene	01.029	92	95.9	95.5–96.2

Potential contaminants are considered as part of the product specification and are monitored as part of the hazard analysis and critical control point (HACCP) 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 and homogeneity

The minimum shelf life of the compounds under assessment range from 6 to 24 months when stored in closed containers under recommended conditions is determined on the basis of compliance with the original specification over this storage period.

Although no data are required for the stability of volatile additives in pre-mixtures and feed, their use in water for drinking introduces other issues relating to product stability, such as degradation due to microbial activity. The FEEDAP Panel notes that all these compounds under assessment have very low water solubilities (Log K_{ow} > 4), which makes it difficult to assess the safety in water for drinking. Considering this, and the absence of data on the short-term stability and homogeneity of the additives in water for drinking, the FEEDAP Panel is not in the position to conclude on the use of the additives in water for drinking.

3.1.3. Conditions of use

The applicant proposes the use of the eight additives in feed or water for drinking for all animal species without withdrawal. The normal use level is 1 mg/kg feed and the high use level is 5 mg/kg feed. No proposals are made for the dose to be used in water for drinking.

3.2. Safety

The assessment of safety is based on the highest use level proposed by the applicant (5 mg/kg complete feed).

3.2.1. Absorption, distribution, metabolism and excretion (ADME) and residue studies

These compounds are readily absorbed from the gastrointestinal tract (WHO, 2005).

Data on the metabolism of myrcene in rats and rabbits indicate that acyclic alkenes (myrcene and β-ocimene) are metabolised via epoxidation of double bonds, ultimately resulting in diols, which can be further conjugated. The principal urinary metabolite following gavage administration of myrcene is myrcene-3,10-glycol, formed from the hydration of the epoxide intermediate in both species. Epoxidation of the 3,10-double bond was favoured over epoxidation of the 1,2-double bond. The studies indicate that the formation of diols from the myrcene-epoxides is very efficient (EFSA CEF Panel, 2015a).

Metabolism data are available for α-pinene, β-pinene, camphene, δ-3-carene and β-caryophyllene (bicyclic, non-aromatic hydrocarbons). In general, the metabolic options for these substances include oxidation of methyl ring substituent groups to give the corresponding alcohol and further oxidation products. For the substances studied, double bond epoxidation has also been demonstrated. In

addition, ring cleavage has also been observed, e.g. for β-pinene resulting in the formation of monocyclic terpenoid derivatives like α-terpineol. Hydroxylated metabolites (i.e. alcohols) or further metabolic products may be eliminated as conjugates e.g. with glucuronic acid (EFSA CEF Panel, 2015a). No specific data are available for the metabolism of valencene.

Little is known about the specific metabolic pathways involved in metabolism of CG 31 compounds in livestock. However, the enzymes involved in the biotransformation pathways of these compounds are present in all the food-producing target species. The cytochrome P450 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), fish (Wolf and Wolfe, 2005) and birds (Blevins et al., 2012). Epoxide hydrolases, the enzymes involved in the detoxication of the epoxides via formation of diols, which are then conjugated and eliminated, are present in mammals (Wisniewski et al., 1987; Marini et al., 1998), fish (Newman et al., 2001) and birds (Harris et al., 2006). In these species (except cats, which have an unusually low capacity for glucuronidation, see Section 3.2.1), conjugation reactions with sulphate and glucuronic acid also take place (Watkins and Klaassen, 1986; James, 1987), producing water-soluble derivatives that are promptly eliminated in urine. Mammals (Watkins and Klaassen, 1986), fish (Espinoza et al. 2013) and birds (Blevins et al., 2012) possess glutathione transferases, which mediate the detoxication of the epoxides by conjugation with glutathione and elimination of the corresponding mercapturic derivatives.

In vitro studies showed that some of the compounds under evaluation (α-pinene, β-pinene, β-ocimene, β-myrcene and δ-3-carene) can undergo degradation when incubated with goat rumen microbiota (Malecky and Broudiscou, 2009; Malecky et al., 2009). After 24-h incubation, the compounds were extensively degraded by the bacteria of the rumen (δ-3-carene to 67%, α- and β-pinene to about 80%, and β-ocimene at 90%). Broudiscou et al. (2007) also studied the degradation of several terpenes in rumen of goats and obtained similar results with an extensive degradation of myrcene, β-ocimene and α-pinene. Haider (2004) investigated the *in vitro* degradation of a variety of essential oil components in bovine rumen content. Among these, some were monoterpenes under assessment (α- and β-pinene, and myrcene). After 24 h of incubation, the monoterpenes disappeared almost completely from the incubation medium (only 10% of β-pinene was recovered). From these studies, it can be concluded that in the rumen some of the compounds under assessment are extensively degraded, with the portion absorbed expected to be low as compared with that administered. The FEEDAP Panel notes that the degradation products were not identified.

The presence of some terpenes in products of animal origin, mainly in milk of grazing cows, has been described by several authors (e.g. Revello Chion et al., 2010). An oily mixture of α-pinene, limonene and β-caryophyllene (1 g each compound/animal per day, corresponding to about 700 mg/kg feed) was orally administered to dairy ewes for 18 days. The three compounds were absorbed, as evidenced by their presence in blood, being the lowest levels obtained for β-caryophyllene along all the experiment, followed by α-pinene (Poulopoulou et al., 2012a). In milk, β-caryophyllene peaked at the 7th day of the experiment (about 13 µg/mL), decreasing thereafter, being about 8 µg/mL at the end of the trial (about 4 µg/mL for α-pinene). Two days after stopping the essential oil administration, both β-caryophyllene and α-pinene were secreted in milk at very low concentrations. In cheese prepared with control milk, α-pinene and limonene were present, but not β-caryophyllene. Both α-pinene and β-caryophyllene were present in the treated samples, although limonene was the most transferred terpene. The same group (Poulopoulou et al., 2012b) performed a study in goats with a similar protocol and found a peak plasma concentration for β-caryophyllene (as well as for limonene) 4 h after the first administration (0.160 µg/mL) and about 0.050 µg/mL for α-pinene that almost disappeared after 24 h (< 0.01 µg/mL). At the 7th day of administration, the plasma levels peaked (about 0.070 µg/mL and 0.050 µg/mL for β-caryophyllene and α-pinene, respectively) decreasing to very low levels (< 0.005 µg/mL) on the 18th day. In milk, α-pinene was present at the highest levels, attaining almost 1.0 µg/mL on the 18th day, decreasing rapidly thereafter (0.4 µg/mL 2 days after stopping the treatment). β-Caryophyllene was not detected in milk (limit of detection of the method not given). α-Pinene and β-caryophyllene were present in cheese samples, prepared with milk from treated goats, being α-pinene also present in control cheese, although at lower concentrations. The authors consider that the dose of the compounds used in these experiments is representative of that consumed by grazing animals.

The transfer of terpenes in milk was also assessed in dairy cows by administering to the animals two types of essential oils (caraway and oregano) through duodenal cannulas and by inhalation (Lejonklev et al., 2013). Some compounds under assessment were present in these products, namely, α-pinene, β-pinene, β-myrcene, δ-3-carene and β-caryophyllene. Although the residues of the compounds in milk were determined in a total basis, there was a significant increase in the total terpene levels in milk obtained immediately after duodenal treatment compared with milk obtained before treatment and from the next morning's milking. Importantly, the total terpene content did not differ in the milk samples collected before treatment and the morning following treatment, showing that the transfer of compounds into the milk is rapid but short-lived. It has to be noted that the terpenes were introduced directly in the duodenum bypassing the degradation by rumen microbiota and that only one administration was made although spread for 9 h.

The accumulation of terpenes in muscle and adipose tissues was investigated in calves (two calves per group) fed milk replacer unsupplemented or supplemented with 5 or 20 µL/L of a mixture of essential oils. The dose increased from 10 µL essential oil/day at the beginning of the study to 40 µL essential oil/day at the end, when animals reached a live weight of about 250 kg (Serrano et al., 2007). There was no evidence of increased monoterpene concentrations in tissues of animals supplemented with essential oils, but an increase in sesquiterpene concentrations was seen in some adipose tissues of one the two treated animals.

3.2.2. Toxicological studies

An Organisation for Economic Co-operation and Development OECD 408 compliant 90-day study with β-caryophyllene (Bauter, 2013a) was assessed by the EFSA CEF Panel (EFSA CEF Panel 2015a). Briefly, four groups of rats (10/sex/dietary intake level) of male and female CRL Sprague–Dawley rats were fed a diet providing daily intakes of 0, 222, 456 and 1,367 mg β-caryophyllene/kg body weight (bw) for males and 0, 263, 1,033 and 4,278 mg β-caryophyllene/kg bw for females, respectively. At the middle and high doses, several adverse effects were observed. Haematological parameters in males showed a dose-dependent increase in white blood cells and several changes in other blood cells. Pathology and histopathology revealed an increase in the absolute and relative liver weight associated with hepatocellular hypertrophy in both sexes, the presence of erythrocytes in the sinuses of the mesenteric lymph nodes in both sexes and an increase in relative kidney weight in females, not associated with microscopically alterations. Based on the toxicological findings in haematology in males, the liver and the mesenteric lymph node pathology in both sexes, and the non-explained effects in female kidneys, the EFSA CEF Panel concluded that only the lowest dose in male rats (222 mg/kg bw per day) provides a no-adverse-effect level (NOAEL) for β-caryophyllene. The FEEDAP Panel supports the conclusions by the CEF Panel.

In an OECD 408 compliant 90-day study with myrcene (Bauter, 2013b), four groups of adult Sprague-Dawley rats (10/sex/group) were maintained on diets providing 8.0, 40 and 44 mg myrcene/kg bw per day for males, and 9.6, 48 and 53 mg myrcene/kg bw per day for females. There were no mortalities, clinical pathological findings, changes in macroscopic or microscopic histopathology, or organ weight changes in the groups administered myrcene during the study. Under the conditions of the study and based on the toxicological endpoints evaluated, the NOAEL for administration of myrcene in the diet was determined to be the highest dose, calculated to provide an estimated daily intake of 44 mg/kg bw per day for males and 53 mg/kg bw per day for females, respectively.

The EFSA CEF Panel decided to accept the NOAEL of this study (44 mg/kg bw per day). The FEEDAP Panel supports the conclusions by the CEF Panel.

3.2.3. Safety for the target species

The first approach to the safety assessment for target species takes account of the applied 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 European Union (EU) to the individual compounds range from 13 to 1800 µg/person per day (EFSA CEF Panel, 2015b). This corresponds to range from 0.6 to 83 µg/kg $bw^{0.75}$ per day. These exposure levels are considered safe for humans. Table 3 summarises the results of the comparison with human exposure for representative target animals. The body weight of target animals is taken from the default values shown in Table 4.

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

Flavouring	Use level in feed (mg/kg)	Human exposure (µg/kg $bw^{0.75}$ per day)(1)	Target animal exposure µg/kg $bw^{0.75}$ per day		
			Salmon	Piglet	Dairy cow
β-Pinene	5	60	118	526	777
α-Pinene	5	83	118	526	777
β-Caryophyllene	5	15	118	526	777
Myrcene	5	13	118	526	777
Camphene	5	0.6	118	526	777
Valencene	5	2.5	118	526	777
β-Ocimene	5	2.6	118	526	777
δ-3-Carene	5	13	118	526	777

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

The data in Table 3 show that for all the eight compounds under assessment the intake by target animals greatly exceeds that of humans, resulting from use in food. As a consequence, 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 (subchronic, repeated-dose studies) were available for β-caryophyllene [01.007] and myrcene [01.008] from which a NOAEL value could be derived (see Section 3.2.2). In the absence of toxicological data, the NOAEL of β-caryophyllene (222 mg/kg bw per day) is considered suitable to derive a safe feed level for β-pinene [01.003], α-pinene [01.004], camphene [01.009] and δ-3-carene [01.029]. Similarly, the NOAEL of myrcene (44 mg/kg bw per day) could be used to derive a safe level in feed for β-ocimene [01.018]. As data on the metabolism of valencene [01.017] were not available, the FEEDAP Panel did not apply the NOAEL of β-caryophyllene to valencene [01.017]. Applying an uncertainty factor (UF) of 100 to the NOAELs, the maximum safe intake for the target species was derived for the compounds following the EFSA Guidance for sensory additives (EFSA, 2012a), and thus the maximum safe feed concentration was calculated (Table 4 and 5). Because glucuronidation of the hydrolysis or oxidation products of the compounds in Table 4 and 5 is an important metabolic reaction to facilitate the excretion of these compounds (see Section 3.2.1), their use as additives in cat feed needs an additional uncertainty factor of 5. This factor was derived from the fact that cats have an unusually low capacity for glucuronidation (Court and Greenblatt, 1997).

Table 4: Maximum safe concentration in feed for different target animals for β-caryophyllene [01.007], β-pinene [01.003], α-pinene [01.004], camphene [01.009] and δ-3-carene [01.029]

Target animal	Default values		Maximum safe intake/feed concentration	
	Body weight (kg)	Feed intake (g/day)(1)	Intake (mg/day)	Concentration (mg/kg feed)(2)
Salmonids	2	40	4	112
Veal calves (milk replacer)	100	2,000	222	111
Cattle for fattening	400	8,000	888	111
Pigs for fattening	100	3,000	222	74
Sows	200	6,000	444	74
Dairy cows	650	20,000	1,443	72
Turkeys for fattening	12	400	27	67
Piglets	20	1,000	44	44
Chickens for fattening	2	120	4	37
Laying hens	2	120	4	37
Dogs	15	250	33	133
Cats	3	60	7	22(3)

(1): Complete feed with 88% 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.
(2): In cattle for fattening, dairy cows, dogs and cats the values are in mg/kg DM intake.
(3): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

Table 5: Maximum safe concentration in feed for different target animals for myrcene [01.008] and β-ocimene [01.018]

Target animal	Default values		Maximum safe intake/feed concentration	
	Body weight (kg)	Feed intake (g/day)(1)	Intake (mg/day)	Concentration (mg/kg feed)(2)
Salmonids	2	40	1	22
Veal calves (milk replacer)	100	2,000	44	22
Cattle for fattening	400	8,000	176	22
Pigs for fattening	100	3,000	44	15
Sows	200	6,000	88	15
Dairy cows	650	20,000	286	14
Turkeys for fattening	12	400	5	13
Piglets	20	1,000	9	9
Chickens for fattening	2	120	1	7
Laying hens	2	120	1	7
Dogs	15	250	7	26
Cats	3	60	1	4(3)

(1): Complete feed with 88% 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.
(2): In cattle for fattening, dairy cows, dogs and cats the values are in mg/kg DM intake.
(3): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

No suitable toxicological study could be identified from which to derive a NOAEL for valencene [01.017]. Therefore, the threshold of toxicological concern (TTC) approach was used to derive the safe feed concentration for this Cramer Class I compound. This provides a value of 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 (EFSA FEEDAP Panel, 2012a).

Conclusions on safety for the target species

The FEEDAP Panel concludes that for:

- β-caryophyllene [01.007], β-pinene [01.003], α-pinene [01.004], camphene [01.009] and δ-3-carene [01.029] the proposed maximum use level (5 mg/kg feed) is safe for all target species, with a margin of safety ranging from 5 to 23.4-fold;
- myrcene [01.008] and β-ocimene [01.018] the proposed maximum use level (5 mg/kg feed) is safe for all target species except cats, with a margin of safety ranging from 1.4 to 4.6-fold. For cats, the calculated maximum safe concentration is 4 mg/kg complete feed.
- valencene [01.017] 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.

3.2.4. Safety for the consumer

The consumers are exposed to the compounds under assessment due to their natural occurrence in food and use as food flavours. The application as flavours in animal feed would further add to this exposure (Section 3.2.1).

The additional exposure of consumers of products from supplemented animals cannot be calculated because much of the available data is qualitative in nature. The only quantitative study identified reported a significant increase in the terpene concentrations in milk from supplemented animals (Pouloupoulou, 2012b). This study involved a dose considerably higher (700 times) than the proposed use level, a dose considered by the authors to be representative of the exposure of grazing animals. Data on tissue deposition are scarce. In a preliminary study made in calves, the concentration of monoterpenes in adipose tissues appeared not to be influenced by supplementation.

The FEEDAP Panel recognises the uncertainties introduced by a weak dataset. However, considering the data on metabolism and toxicity of the compounds under consideration and the proposed use levels, the Panel concludes that supplementation of animals diets with any of the eight compounds under assessment would not raise concerns for consumer safety.

3.2.5. Safety for the user

No specific data on the safety for the user were provided. In the material safety data sheets[9] 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. No specific data on sensitisation were provided.

3.2.6. Safety for the environment

The compounds are naturally present in the environment and metabolised by the target species (see Section 3.2.1) and expected to be largely excreted as metabolites. Therefore, it is considered that the use of the compounds in animal nutrition would not pose a risk for the environment provided that the concentrations regarded as safe for the target species are not exceeded.

3.3. Efficacy

As all eight 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.

The FEEDAP Panel does not have information on the possible influence of the compounds on the sensory properties of milk and milk products.

4. Conclusions

The FEEDAP Panel concludes that β-pinene [01.003], α-pinene [01.004], β-caryophyllene [01.007], myrcene [01.008], camphene [01.009], β-ocimene [01.018] and δ-3-carene [01.029] are safe at the proposed maximum dose level (5 mg/kg complete feed) for all animal species, except myrcene and beta-ocimene when 4 mg/kg would apply for cats. For valencene, 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.

No safety concern would arise for the consumer when the compounds are used at the safe levels identified.

The Panel is unable to conclude on user safety in the absence of specific data.

The use of the compounds in animal nutrition would not pose a risk for the environment provided that the concentrations regarded as safe for the target species are not exceeded.

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.

In the absence of data on the stability/survival in water for drinking, the FEEDAP Panel is unable to conclude on the safety or efficacy of the substances under this mode of delivery.

[9] Technical dossier/Section II/Annex II.3

Documentation provided to EFSA

1. Chemically defined flavourings from Flavouring Group 31. June 2010. Submitted by FEFANA asbl./Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

2. Chemically defined flavourings from Flavouring Group 31. Supplementary information May 2012. Submitted by FEFANA asbl./Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

3. Chemically defined flavourings from Flavouring Group 31. Supplementary information January 2013. Submitted by FEFANA asbl./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 flavourings from Flavouring Group 31.

5. Comments from Member States

References

Bauter MR, 2013a. β-Caryophyllene: a 90-day dietary study in rats. Product Safety labs. Study no. 33328. January 7, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bauter MR, 2013b. Myrcene: a 90-day dietary study in rats. Product Safety labs. Study no. 33456. May 20, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat

Blevins S, Siegel PB, Blodgett DJ, Ehrich M and Lewis RM, 2012. Liver enzymes in White Leghorns selected for the sheep red blood cell immune response. Poultry Science, 91, 322–326.

Broudiscou LP, Cornu A and Rouzeau A, 2007. *In vitro* degradation of 10 mono- and sesquiterpenes of plant origin by caprine rumen microorganisms. Journal of the Science of Food and Agriculture, 87, 1653–1658.

Court MH and Greenblatt DJ, 1997. Molecular basis for deficient acetaminophen glucuronidation in cats. Biochemical Pharmacology, 53, 1041–1047.

EFSA (European Food Safety Authority), 2008. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008, 842, pp. 1–28.

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015a. Scientific Opinion on Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3): Aliphatic and aromatic hydrocarbons from chemical group 31. EFSA Journal 2015; 13(4):4069, 115 pp. doi:10.2903/j.efsa.2015.4069

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015b. Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2. EFSA Journal 2015; 13(4):4067, 72 pp. doi:10.2903/j.efsa.2015.4067

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for sensory additives. EFSA Journal 2012;10(1):2534, 26 pp. doi:10.2903/j.efsa.2012.2534

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for the preparation of dossiers for additives already authorised for use in food. EFSA Journal 2012;10(1):2538, 4 pp. doi:10.2903/j.efsa.2012.2538

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. doi:10.2903/j.efsa.2012.2537

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012d. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety and efficacy of aliphatic and aromatic hydrocarbons (chemical group 31) when used as flavourings for all animal species. EFSA Journal 2015; 13(5):4053, 22 pp. doi:10.2903/j.efsa.2015.4053

Espinoza HM, Shireman LM, McClain V, Atkins W and Gallagher EP, 2013. Cloning, expression and analysis of the olfactory glutathione S-transferases in coho salmon. Biochemical Pharmacology, 85, 839–848.

Haider V, 2004. Untersuchungen zum Verhalten von Monoterpenen im Mageninhaltd des Rindes. Dissertation (Ph.D. Thesis), Vet. Med. Univ. Wien, pp. 90.

Harris TR, Morisseau C, Walzem RL, Ma SJ, and Hammock BD, 2006. The cloning and characterization of a soluble epoxide hydrolase in chicken. Poultry Science, 85, 278–287.

Ioannides C, 2006. Cytochrome P450 expression in the liver of food-producing animals. Current Drug Metabolism, 7, 335–348.

James MO, 1987. Conjugation of organic pollutants in aquatic species. Environmental Health Perspectives, 71, 97–103.

Lejonklev J, Løkke MM, Larsen MK, Mortensen G, Agerlin M and Weisbjerg MR, 2013. Transfer of terpenes from essential oils into cow milk. Journal of Dairy Science, 96, 4235–4241.

Malecky M, and Broudiscou LP, 2009. Disappearance of nine monoterpenes exposed in vitro to the rumen microflora of dairy goats: Effects of inoculum source, redox potential, and vancomycin. Journal of Animal Science, 87, 1366–1373.

Malecky M, Fedele V, and Broudiscou LP, 2009. In vitro degradation by mixed rumen bacteria of 17 mono- and sesquiterpenes typical of winter and spring diets of goats on Basilitica rangelands (southern Italy). Journal of the Science of Food and Agriculture, 89, 531–536.

Marini S, Longo V, Mazzacaro A and, Gervasi PG, 1998. Xenobiotic-metabolizing enzymes in pig nasal and hepatic tissues. Xenobiotica, 28, 923–935.

Nebbia C, Dacasto M, Rossetto Giaccherino A, Giuliano Albo A and Carletti M, 2003. Comparative expression of liver cytochrome P450-dependent monooxygenases in the horse and in other agricultural and laboratory species. The Veterinary Journal, 165, 53–64.

Newman JW, Denton DL, Morisseau C, Koger CS, Wheelock CE, Hinton DE and Hammock BD, 2001. Evaluation of fish models of soluble epoxide hydrolase inhibition. Environmental Health Perspectives, 109, 61–66.

Poulopoulou I, Zoidis E, Massouras T and Hadjigeorgiou I, 2012a. Terpenes transfer to milk and cheese after oral administration to sheep fed indoors. Journal of Animal Physiology and Animal Nutrition, 96, 172–181.

Poulopoulou I, Zoidis E, Massouras T and Hadjigeorgiou I, 2012b. Transfer of orally administered terpenes in goat milk and cheese. Journal of Animal Science, 25, 1411–1418.

Revello Chion A, Tabacco E, Giaccone D, Peiretti PG, Battelli G and Borreani G, 2010. Variation of fatty acids and terpene profiles in mountain milk and "Toma piemontese" cheese as affected by diet composition in different seasons. Food Chemistry, 121, 393–399.

Serrano E, Cornu A, Kondjoyan N, Figueredo G, Agabriel J and Micol D, 2007. Terpene accumulation in muscle and fatty tissues of calves supplemented with essential oils. Journal of Animal and Feed Sciences, 16, 168–179.

Watkins JB III and Klaassen CD, 1986. Xenobiotic biotransformation in livestock: comparison to other species commonly used in toxicity testing. Journal of Animal Science, 63, 933–942.

WHO (World Health Organization), 2005. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives, 8–17 June 2004, Geneva, Switzerland. World Health Organization Technical Report Series 928.

Wisniewski JA, Moody DE, Hammock BD and Shull LR, 1987. Interlobular distribution of hepatic xenobiotic-metabolizing enzyme activities in cattle, goats and sheep. Journal of Animal Science, 64, 210–215.

Wolf JC and Wolfe MJ, 2005. A brief overview of nonneoplastic hepatic toxicity in fish. Toxicologic Pathology, 33, 75–85.

Abbreviations

ADI	average daily intake
ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CDG	chemically defined group
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
DM	dry matter
EC	European Commission
EU	European Union
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FFAC	Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures)
FGE	food group evaluation
FLAVIS	EU Flavour Information System
FL-no	FLAVIS number
GC–MS	gas chromatography–mass spectrometry
GC–MS RTL	gas chromatography–mass spectrometry using retention time locking
HACCP	hazard analysis and critical control points
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
Log K_{ow}	logarithm of octanol–water partition coefficient
$bw^{0.75}$	metabolic body weight
MSDI	maximised survey-derived daily intake
MW	molecular weight
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
RTL	retention time locking
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 Aliphatic and aromatic hydrocarbons[10]

The *Chemically Defined Flavourings - Group 31 (CDG31, Aliphatic and aromatic hydrocarbons)*, in this application comprises 17 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 90% to 99% (80% for β-Caryophyllene, Camphene and β-Ocimene).

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 CDG31 in the *feed additives*, the Applicant submitted a qualitative multi-analyte 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 CRL. The Applicant provided the typical chromatogram for the *CDG31* 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. As the substances of CDG31 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 CDG31 in the mixture of flavouring compounds.

Based on the satisfactory experimental evidence provided, the CRL recommends for official control for the qualitative identification in the feed additive of the individual (or mixture of) flavouring compounds of interest listed in Table 1 the GC–MS RTL (Agilent specific) method submitted by the Applicant.

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 CRL is unable to recommend a method for the official control to identify the active substance(s) of interest listed in Table 1 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.

[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2010-0022?search&form-return