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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of aliphatic and alicyclic ethers (chemical group 16) when used as flavourings for all animal species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2, 3]

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ABSTRACT

Chemical group 16 consists of aliphatic and alicyclic ethers, of which four are currently authorised for use as flavours in food. The FEEDAP Panel was unable to perform an assessment of 1,5,5,9-tetramethyl-13-oxatricyclo[8.3.0.0.(4.9)]tridecane and theaspirane because of issues related to the purity of the compounds. The FEEDAP Panel concludes that: i)1,8-cineole is safe at the high use level proposed by the applicant (5 mg/kg complete feed) for all animal species with a margin of safety of 5.6 to 28.2; ii)2-(2-methylprop-1-enyl)-4-methyltetrahydropyran (Class II) is safe at a maximum of 0.3 mg/kg complete feed for cattle, salmonids and non food producing animals and of 0.5 mg/kg complete feed for pigs and poultry. The absence of a margin of safety would not allow the simultaneous administration in feed and water for drinking of these substances. The total dose from all sources should not exceed that recommended when given in feed alone. No safety concern would arise for the consumer from the use of compounds belonging to CG 16 up to the highest safe level in feedingstuffs for all animal species. The FEEDAP Panel considers it prudent to treat both compounds under assessment as irritants to skin, eyes and respiratory tract, and as skin sensitisers. The FEEDAP Panel considers that the concentrations of the compounds belonging to CG 16 in the environment are not expected to exceed levels of concern when used in animal feeds at the levels considered to be safe to the target species. Since these 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.

KEY WORDS

Sensory additives, flavourings, aliphatic and alicyclic ethers, chemical group 16, 1,8-cineole, 2-(2-methylprop-1-enyl)-4-methyltetrahydropyran, 1,5,5,9-tetramethyl-13-oxatricyclo[8.3.0.0.(4.9)]tridecane, theaspirane

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[1] On request from the European Commission, Question No EFSA-Q-2010-00989, adopted on 13 November 2012.

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SUMMARY

Following a request from the European Commission, the 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 four compounds (aliphatic and alicyclic ethers belonging to chemical group 16) when used as flavourings for all animal species. All are currently authorised for use as flavours in food and have all been detected in plant materials, or in processed foods, however the reports of their distribution vary greatly.

The FEEDAP Panel was unable to perform an assessment of 1,5,5,9-tetramethyl-13-oxatricyclo[8.3.0.0.(4.9)]tridecane and theaspirane because of issues related to the purity of the compounds.

The FEEDAP Panel concludes that:

- 1,8-cineole is safe at the high use level proposed by the applicant (5 mg/kg complete feed) for all animal species with a margin of safety of 5.6 to 28.2

- 2-(2-methylprop-1-enyl)-4-methyltetrahydropyran (Class II) is safe at a maximum of 0.3 mg/kg complete feed for cattle, salmonids and non food producing animals and of 0.5 mg/kg complete feed for pigs and poultry. The absence of a margin of safety would not allow the simultaneous administration in feed and water for drinking of these substances.

The total dose from all sources should not exceed that recommended when given in feed alone.

No safety concern would arise for the consumer from the use of compounds belonging to CG 16 up to the highest safe level in feedingstuffs for all animal species.

The FEEDAP Panel considers it prudent to treat both compounds under assessment as irritants to skin, eyes and respiratory tract, and as skin sensitisers.

The FEEDAP Panel considers that the concentrations of the compounds belonging to CG 16 in the environment are not expected to exceed levels of concern when used in animal feeds at the levels considered to be safe to the target species.

Since these 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.

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BACKGROUND

Regulation (EC) No 1831/2003[4] 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, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[5] for authorisation of 1,8-cineole, 2-(2-methylprop-1-enyl)-4-methyltetrahydropyran, 1,5,5,9-tetramethyl-13-oxatricyclo[8.3.0.0.(4.9)]tridecane, theaspirane (Table 1) belonging to chemical group 16, aliphatic and alicyclic ethers to be used as feed additives for all animal species (category: sensory additives; functional group: flavourings) under the conditions mentioned in Table 1.

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.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 24 August 2010.

The additives are listed as food and feed flavourings in the register of Flavouring substances[7] and in the European Union Register of Feed Additives, respectively.

The four substances belonging to CG 16 have been previously assessed by JECFA (2004a,b) and EFSA (2008a, 2011a,b) as food flavourings. They have not been previously assessed by EFSA as feed additives.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 the efficacy of 1,8-cineole, 2-(2-methylprop-1-enyl)-4-methyltetrahydropyran, 1,5,5,9-tetramethyl-13-oxatricyclo[8.3.0.0.(4.9)]tridecane, theaspirane , when used under the conditions described in Table 1.

[4] 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.

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

[6] EFSA Dossier reference: FAD-2010-0042.

[7] 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 Text with EEA relevance. OJ L 267, 2.10.2012, p. 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Chemical defined flavourings from Chemical Group 16: 1,5,5,9-Tetramethyl-13-oxatricyclo [8.3.0.0.(4.9)]tridecane 1,8-Cineole 2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran Theaspirane
Registration number/EC No/No (if appropriate)	-
Category of additive	2. Sensory additives
Functional group of additive	b) flavouring compounds

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis[8] (if appropriate)
1,5,5,9-Tetramethyl-13-oxatricyclo [8.3.0.0.(4.9)]tridecane (CAS No 3738-00-9)	$C_{16}H_{28}O$	96 %	NMR
1,8-Cineole (CAS No 470-82-6)	$C_{10}H_{18}O$	98 %	IR
2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran (CAS No 16409-43-1)	$C_{10}H_{18}O$	99 %	NMR
Theaspirane (CAS No 36431-72-8)	$C_{13}H_{22}O$	97 %	NHMR IR MS

Trade name (if appropriate)	-
Name of the holder of authorisation (if appropriate)	-

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg or Units of activity or CFU/kg of complete feedingstuffs (select what applicable)		
All species and categories	-	-	-	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	-
Specific conditions or restrictions for handling (if appropriate)	All feedingstuffs and water for drinking, as part of a premixture only
Post-market monitoring (if appropriate)	-
Specific conditions for use in complementary feedingstuffs (if appropriate)	-

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues

[8] Available at the webpage of the EURL.

ASSESSMENT

1. Introduction

The Chemical Group (CG) 16 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[9] as 'aliphatic and alicyclic ethers'. The present application concerns four compounds, which can be assigned to this CG. The flavours included in this assessment are distributed in plant materials.

All four compounds have been previously assessed by JECFA (2004a,b) and EFSA (2008a, 2011a,b) and were considered safe for use as flavours in food. No Acceptable Daily Intake (ADI) values were specified. The four compounds are currently listed in the European Union database of flavouring substances and as such authorised for use in food in the European Union.

A consortium of companies (FFAC) supplying flavours to the feed industry has requested authorisation for the use of the substances listed in Table 2 as additives to feed and water for drinking (category: sensory additives, flavouring compounds) for use in all animal species.

Regulation (EC) No 429/2008[10] 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. Characterisation

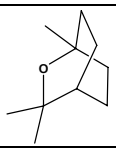
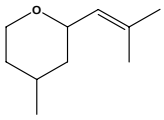
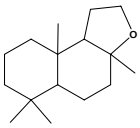
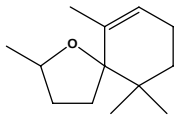
2.1. Characterisation of the flavouring additives

The molecular structures and the physico-chemical characteristics of the additives under application are summarised in Table 2.

[9] 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.

[10] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1-65.

Table 2: Chemically defined flavourings of CG 16 under application

Flavouring	1,8-Cineole (Eucalyptol)	2-(2-Methylprop-1-enyl)-4-methyl tetrahydropyran	1,5,5,9-Tetramethyl-13-oxatricyclo [8.3.0.0.(4.9)]tridecane	Theaspirane
CAS No.	470-82-6	16409-43-1	3738-00-9	36431-72-8
FLAVIS No.	03.001	13.037	13.072	13.098
Structural formula				
Molecular formula	$C_{10}H_{18}O$	$C_{10}H_{18}O$	$C_{16}H_{28}O$	$C_{13}H_{22}O$
Molecular weight	154.25	154.25	236.4	194.32
Physical status	Liquid	Liquid	Solid	Liquid
Log K_{ow}	2.74	3.58*	4.76*	4.79

* KowWin Estimate

All four substances except 1,8-cineole which is obtained by distillation from *Eucalyptus globulus* are produced by chemical synthesis. The various routes of synthesis are described in the dossier.[11]

Data were provided on the batch to batch variation in five batches of each additive.[12] The content of the active substance exceeded the JECFA specifications (JECFA, 2006) for 1,8-cineole and 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran. One batch of 1,5,5,9-tetramethyl-13-oxatricyclo [8.3.0.0.(4.9)]tridecane and two batches of theaspirane exceeded JECFA specifications, the remaining batches 'reflecting the use from industry' were characterised by lower purity (Table 3). This description does not allow the setting of specification or the extrapolation of consumer safety assessments of these substances. Consequently, these additives are excluded from further consideration.

Table 3: Identification of the substances and data on purity

EU Register name	JECFA specification %	Assay %	
		Average	Range
1,8-Cineole (Eucalyptol)	> 98	99.5	98.8–99.8
2-(2-Methylprop-1-enyl)-4-methyl tetrahydropyran	> 99	99.4	99.0–99.8
1,5,5,9-Tetramethyl-13-oxatricyclo [8.3.0.0.(4.9)]tridecane	> 96	88.4	81.2–99.8
Theaspirane	> 97*	94.5	90.7–97.1

* Sum of stereoisomers

Potential contaminants are considered as part of the product specification and are monitored as part of the HACCP procedure applied by all consortium members. The parameters considered include residual solvents, heavy metals and other undesirable substances.

2.2. Stability

A shelf life of at least 12 to 24 months is given for these chemicals when stored in closed containers under recommended conditions (in a cool and dry place). This assessment is made on the basis of compliance with the original specification after storage.

[11] Technical dossiers/Section II.
[12] Technical dossiers/Section II/Annex 2.1 and Supplementary Information May 2011.

Although no data is required for the stability of volatile additives in premixes and feed, 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 products in CG 16 have low water solubility (Log K_{ow} >2) which makes it difficult to assess the safety in water for drinking.

No data on the short term stability of the additive in water for drinking were provided; the FEEDAP Panel is therefore not in the position to comment on this route of administration.

2.3. Conditions of use

The applicant proposes the use of the two additives in feed or water for drinking for all animal species without withdrawal. In each case the applicant proposes a normal use level of 1 mg/kg and a high use level of 5 mg/kg complete feed. No specific proposals are made for the doses used in water for drinking.

2.4. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of Chemically Defined Flavourings – Group 16 (CG16 – Aliphatic and alicyclic ethers) in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

The assessment of safety is based on the high use level proposed by the applicant (5 mg/kg complete feed) for 1,8-cineole and 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran.

3.1. 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. The data for human exposure in the EU (EFSA, 2008a) range between 0.85 and 1200 µg/person/day, which equates to 0.04 and 55.7 µg/mbw ($kg^{0.75}$) per day. Table 5 summarises the result 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 5.

Table 4: Comparison of exposure of humans and target animals to the flavourings under application

Flavouring	Use level in feed (mg/kg)	Human exposure µg/mbw ($kg^{0.75}$)/day*	Target animal exposure µg/mbw ($kg^{0.75}$)/day		
			Salmon	Piglet	Dairy cow
1,8-Cineole (Eucalyptol)	5	55.7	118	526	777
2-(2-Methylprop-1-enyl)-4-methyl tetrahydropyran	5	0.04	118	526	777

* mbw = metabolic body weight ($kg^{0.75}$) for a 60 kg person = 21.6

The data in Table 5 clearly indicate that the intake by the target animals exceeds that of humans, resulting from use in food for the two compounds. 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 as safe for the target animal can be derived from the lowest No Observed Adverse Effect Level (NOAEL) when suitable data is available. Toxicological data (sub-chronic studies) was found for the two compounds.

For 1,8-cineole (eucalyptol) a NOAEL of 562.5 mg/kg bw per day was identified in a 28-day study in mice (doses: 0, 3750, 7500, 15 000 and 30 000 mg/kg equivalent to approximately 0, 562.5, 1125, 2250 and 4500 mg/kg bw per day, administration route: diet). No effects on mortality, feed and water consumption, body weight, organ weight, gross pathology and histopathology were observed up to the highest dose tested (NTP, 1987; 1987a). The liver weight:body weight ratios of male mice at the three higher doses were significantly higher than those of controls and of animals at the lowest dose (562.5 mg/kg bw per day). A minimal, but dose-related, hypertrophy of the centrilobular hepatocytes was reported in males receiving encapsulated eucalyptol at the three higher doses (control, 0/6; 562.5 mg/kg bw per day, 0/6; 1125 mg/kg bw per day, 1/6; 2250 mg/kg bw per day, 5/6; 4500 mg/kg bw per day, 6/6) and in females at the two higher doses (control, 0/6; 562.5 mg/kg bw per day, 1/6; 1125 mg/kg bw per day, 0/6; 2250 mg/kg bw per day, 4/6; 4500 mg/kg bw per day, 6/6).

Applying a safety factor of 100 (10 for interspecies variations and 10 for intraspecies variations) to this NOAEL and an additional factor of 2 because of the short duration of the study, a maximum safe intake and thus the maximum safe feed concentrations were derived for 1,8-cineole for the different target species following the EFSA Guidance for sensory additives (EFSA, 2012). The results of the calculations are shown in Table 5.

Because glucuronidation of the hydrolysis or oxidation products of the compounds in Table 4 is an important metabolic reaction to facilitate the excretion of these compounds (see section 3.2), their use as additives in cat feed needs an additional safety factor of 5. This factor was derived from the fact that cats have an unusually low capacity for glucuronidation (Court and Greenblatt, 1997).

Table 5: Derived maximum safe concentration in feed for different target animals for 1,8-cineole belonging to CG 16

	Default settings		Maximum safe intake/feed concentration	
Target animal	**Body weight (kg)**	**Feed intake (g/d)**	**Intake (mg/d)**	**Concentration (mg/kg feed)**
Salmonids	2	40	6	141
Veal calves (milk replacer)	100	2000	281	141
Cattle for fattening	400	8000	1125	141
Pigs for fattening	100	3000	281	94
Sows	200	6000	563	94
Dairy Cows	650	20000	1828	91
Turkeys for fattening	12	400	34	84
Piglets	20	1000	56	56
Chickens for fattening	2	120	6	47
Laying hens	2	120	6	47
Dogs	15	250	42	169
Cats	3	60	2	28*

* The safety factor for cats is increased by an additional factor of five because of the reduced capacity of glucuronidation in this species.

In a 90-day study, rats (10-16 M/F) were treated with 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran (a study with only one dose: 2.5 and 2.8 mg/kg bw per day for males and females, respectively, by gavage). No effects on growth, food intake, haematological and clinical chemistry parameters, organ weight or organ pathology were observed at the dose tested (Posternak et al., 1969). This study was not considered suitable for setting a NOAEL because it consisted of one low dose only.

Instead the threshold of toxicological concern (TTC) approach was used to derive the maximum safe concentration in feed for this Cramer Class II compound. The calculated safe use level for this compound (2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran) is 0.3 mg/kg complete feed for cattle, salmonids and non food-producing animals and 0.5 mg/kg complete feed for pigs and poultry.[13]

3.1.1. Conclusions on the safety for target species

The FEEDAP Panel concludes that:

- 1,8-cineole is safe at the high use level proposed by the applicant (5 mg/kg complete feed) for all animal species with a margin of safety of 5.6 to 28.2

- 2-(2-methylprop-1-enyl)-4-methyltetrahydropyran (Class II) is safe at a maximum of 0.3 mg/kg complete feed for cattle, salmonids and non food-producing animals and of 0.5 mg/kg complete feed for pigs and poultry. The absence of a margin of safety would not allow the simultaneous administration in feed and water for drinking of these substances.

The total dose from all sources should not exceed that recommended when given in feed alone.

3.2. Safety for the consumer

The safety for the consumer of these compounds used as food flavours has already been assessed by JECFA (2004) and EFSA (2008a and 2011a,b). All compounds are currently authorised as food additives without limitations.

As the intake of the two compounds by target animals exceeds that of humans resulting from use in food by one to three orders of magnitude, the metabolic fate and potential transfer of significant amounts of residues in edible tissues and products has to be considered.

In its evaluation on aliphatic ethers, either open-chain or cyclic, JECFA (2004) recognised that these compounds are rapidly absorbed from the gastrointestinal tract and are expected to be metabolised by common pathways of metabolism. Alicyclic ethers can be expected to undergo either ring hydroxylation or side chain oxidation (by cytochrome P450 isoenzymes) followed by conjugation with glucuronic acid and excretion in the urine (JECFA, 2004). In humans and other animals, alicyclic ethers such as 1,8-cineole have been shown to be oxidised via P450 isoenzymes to yield polar hydroxylated metabolites, which are conjugated and excreted or further oxidised and excreted. Cleavage of the ether is, at most, a very minor metabolic pathway (Hiroi et al., 1995; Miyazawa et al., 2001a; Miyazawa et al., 2001b; Miyazawa and Shindo, 2001).

The metabolism of 1,8-cineole has been reviewed in the rat, rabbit and human (EFSA, 2011b). It has been reported that 1,8-cineole principally undergoes ring-hydroxylation to form 2- or 3-hydroxy-1,8-cineole, which are subsequently excreted as the glucuronic acid conjugates (Williams, 1959). Indeed, following the gavage administration of 800 mg 1,8-cineole/kg bw to male albino rats, major metabolites included 2- and 3-hydroxy-1,8-cineole and their conjugates and 1,8-dihydroxy-10-carboxy-p-menthane, which was hypothesised to be formed by the oxidation of the metabolite p-

[13] http://www.efsa.europa.eu/en/efsajournal/doc/2534.pdf

menthane-1,8-diol formed by cleavage of the ether linkage (Madyastha and Chadha, 1986). These results are consistent with a more recent study investigating the metabolism of 1,8-cineole in microsomes from male Hooded Wistar rats and humans. In both rats and humans oxidation was preferred at the aliphatic ring carbons over methyl substituents, 2- and 3-hydroxy-1,8-cineole being the major metabolites in rat and human liver microsomes (Pass et al., 2001). The metabolism of 1,8-cineole was studied *in vivo* in rabbits treated by gavage with 200 mg/kg bw. The major metabolites were identified as 2- and 3-hydroxy-1,8-cineole (Miyazawa et al., 1989). When rat and human liver microsomes and recombinant human CYPs were incubated *in vitro* with 1,8-cineole, it was oxidised at high rates to 2-exo-hydroxy-1,8-cineole (Miyazawa et al., 2001b; Miyazawa and Shindo, 2001). As indicated by results obtained with recombinant CYPs, P450 inducers, and specific P450 inhibitors, the reaction in humans is mainly catalysed by CYP3A iso-enzymes (Miyazawa et al., 2001a; Miyazawa et al., 2001b). Furthermore in rats 1,8-cineole is an inducer of CYP2B1 and 3A2 isoenzymes (Hiroi et al., 1995).

Phase I oxidation reactions also plays a significant role in detoxification processes in fish (Di Giulio and Hinton 2008) as well as in birds (Pan and Fouts, 1978). The occurrence of phase II reactions including conjugation with glucuronic acid has been documented in birds (Pan and Fouts, 1978) and fish species (Jobling, 1994). Therefore, in addition to mammals, fish and birds can also be assumed to have the ability to safely metabolize and excrete the flavouring substances from CG 16.

3.2.1. Conclusions on the safety for the consumer

Aliphatic and alicyclic ether are rapidly absorbed, distributed, metabolised and excreted. Mammals, birds and fish share a similar metabolic capacity to handle these compounds. Due to the digestion metabolism and excretion of these compounds by the target species, it is expected that food residues of the CG 16 compounds will give consumer exposures that are considerably less than the levels given to the target species. As the exposure of target species are considered to be safe, the much lower exposure of consumer is also considered to be safe. Metabolites are likely to be of lower toxicity than the parent compounds.

Consequently, no safety concern would arise for the consumer from the use of these compounds up to the highest safe level in feeds.

3.3. Safety for the user

No experimental data on the safety for the user was provided. In the material safety data sheets hazards for skin and eye contact and respiratory exposure are recognised for both compounds under assessment.[14] 1,8-Cineole is identified as 'irritating to the respiratory system' 1,8-cineole and 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran are classified as irritating to eyes and/or skin.

The FEEDAP Panel considers it prudent to treat both compounds under assessment as irritants to skin, eyes and respiratory tract and as skin sensitisers.

3.4. Safety for the environment

1,8-Cineole is present at high concentrations in sage and other plants native to Europe. Therefore, it is not foreseen that the use of 1,8-cineol in animal feeds will substantially increase its concentration in the environment and no further assessment is required. In contrast, the distribution of 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran in the natural environments in the European Union is not known to the FEEDAP Panel and it must therefore be fully assessed through the procedures set out in the Guidance (EFSA, 2008).

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

Based on structure, degradation of 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran in soil and water is predicted (BIOWIN 4.1) to be relatively slow with DT_{50} estimates ranging from days to weeks. It is predicted (KOCWIN) to be moderately mobile with a K_{oc} estimates of 269 dm^3/kg. The compound is predicted (BCFBAF 3.1) to have a low potential for bioaccumulation with bioconcentration factor (BCF) estimates of <300.

Based on a worst case scenario and the highest proposed use level of 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran of 1 mg/kg total feed, the predicted environmental concentrations (PEC) exceed trigger values in all compartments of concern for the environmental risk assessment. PEC_{soil} estimates ranged from 0.004 to 0.018 mg/kg, $PEC_{porewater}$ from 0.50 to 0.96 µg/L and $PEC_{surfacewater}$ from 0.17 to 0.32 µg/L, depending on target species.

No experimental data was found on the toxicity of 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran to species of environmental relevance. Prediction of environmental toxicity based on structure (ECOSAR 1.1) suggested that 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran may be most toxic to fish with an estimated 96-h LC_{50} of 0.65 mg/L. A NOEC of 0.65 µg/L was derived by applying a safety factor of 1000. This concentration is higher than the worst case PEC for surface water and similar to that calculated for porewater.

Considering that worst case scenario PECs for the aquatic compartment is lower than or similar to the predicted NOECs for 2-(2-methylprop-1-enyl)-4-methyl tetrahydropyran this compound is not expected to pose a risk to the environment when used at an inclusion level of 1 mg/kg complete feed. In addition, both compounds considered in this opinion are, as described in Section 3.3, readily absorbed and metabolised in the mammalian target species, thus, adding to the confidence in this conclusion.

4. Efficacy

Since these 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.

CONCLUSIONS

The FEEDAP Panel was unable to perform an assessment of 1,5,5,9-tetramethyl-13-oxatricyclo[8.3.0.0.(4.9)]tridecane and theaspirane because of issues related to the purity of the compounds.

The FEEDAP Panel concludes that:

- 1,8-cineole is safe at the high use level proposed by the applicant (5 mg/kg complete feed) for all animal species with a margin of safety of 5.6 to 28.2

- 2-(2-methylprop-1-enyl)-4-methyltetrahydropyran (Class II) is safe at a maximum of 0.3 mg/kg complete feed for cattle, salmonids and non food producing animals and of 0.5 mg/kg complete feed for pigs and poultry. The absence of a margin of safety would not allow the simultaneous administration in feed and water for drinking of these substances.

The total dose from all sources should not exceed that recommended when given in feed alone.

No safety concern would arise for the consumer from the use of compounds belonging to CG 16 up to the highest safe level in feedingstuffs for all animal species.

The FEEDAP Panel considers it prudent to treat both compounds under assessment as irritants to skin, eyes and respiratory tract, and as skin sensitisers.

The FEEDAP Panel considers that the concentrations of the compounds belonging to CG 16 in the environment are not expected to exceed levels of concern when used in animal feeds at the levels considered to be safe to the target species.

Since these 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.

DOCUMENTATION PROVIDED TO EFSA

1. Chemically defined flavourings from Flavouring Group 16 – Aliphatic and alicyclic ethers (CDG 16). July 2010. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

2. Chemically defined flavourings from Flavouring Group 16 – Aliphatic and alicyclic ethers (CDG 16). Supplementary information. May 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

3. Chemically defined flavourings from Flavouring Group 16 – Aliphatic and alicyclic ethers (CDG 16). Supplementary information. January 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

4. Chemically defined flavourings from Flavouring Group 16 – Aliphatic and alicyclic ethers (CDG 16). Supplementary information. June 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Chemically Defined Flavourings – Group 16 (CDG16 Aliphatic and alicyclic ethers).

6. Comments from Member States received through the ScienceNet.

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APPENDIX

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 16 (CDG16, Aliphatic and alicyclic esters)[15]

The *Chemically Defined Flavourings - Group 16* (*CDG16 - Aliphatic and alicyclic ethers*), in this application comprises four 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 96 % to 99 %.

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 *CDG16* in the *feed additive,* 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 *CDG16* 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 *CDG16* 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 *CDG16* 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 (1,8-Cineole, 2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran, Theaspirane and 1,5,5,9-Tetramethyl-13-oxatricyclo[8.3.0.0.(4.9)]tridecane) 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 in *feedingstuffs* or *water*.

[15] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0042.pdf

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.

GLOSSARY

ADI	acceptable daily intake
bw	Body weight
BCF	Bio Concentration Factor
BCFBAF	Component program of Episuite
BIOWIN	Component program of Episuite
CAS	Chemical Abstract Service
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CD	Commission Decision
CG	Chemical Group
CDG	Chemically Defined Group
CYPs	Cytochromes P450
DM	Dry matter
DT_{50}	Time to 50 % Degradation
EC	European Commission
EC	European Community
EC_{50}	The concentration of a test substance which results in 50 % of the test animals being adversely affected, i.e., both mortality and sub-lethal effects
ECOSAR	Component program of Episuite
EEC	European Economic Community
EEIG	European Economic Interest Group
EFSA	European Food Safety Authority
EPI suite	Estimation Programs Interface (EPI) Suite TM
EU	European Union
EURL	European Union Reference Laboratory
FAO	Food Agricoltural Organisation
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FFAC	Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures)
FGE	Food Group Evaluation
FLAVIS	The EU Flavour Information System
GC – MS	Gas Chromatography – Mass Spectrometry
HACCP	Hazard Analysis and Critical Control Points
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
Koc	Sorption/desorption coefficient, normalized to organic carbon content
Kow	n-Octanol/water partitioning coefficient
KOWIN	Component program of Episuite
LC_{50}	The concentration of a test substance which results in a 50 % mortality of the test species
Log K_{ow}	Log Octanol-Water Partitioning Coefficient (log K_{ow})
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOEL	No observed effect level
PEC	Predicted Environmental Concentrations
SF	Safety Factor
TTC	Threshold of toxicological concern
WHO	World Health Organisation