SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 20 October 2015

PUBLISHED: 10 November 2015

doi:10.2903/j.efsa.2015.4268

Safety and efficacy of saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols belonging to chemical group 5 when used as flavourings for all animal species

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

Abstract

This opinion considers the safety and efficacy of 17 compounds belonging to chemical group 5 when used as feed flavourings for all animal species. Isopropanol and isopropyl tetradecanoate are safe at a maximum dose level of 25 mg and 5 mg/kg feed, respectively, based on comparable levels of exposure of humans and animals to isopropanol. From toxicological data, heptan-2-one and pentan-2-one are safe at the proposed maximum dose level (5 mg/kg) for all species except piglets (4 mg/kg), chickens for fattening and laying hens (3 mg/kg) and cats (2 mg/kg). Since no acceptable toxicological data are available for the remaining compounds, the threshold of toxicological concern approach was used to calculate maximum safe concentrations in feed. For octan-2-ol, pentan-2-ol, octan-3-ol and butan-2-one the safe level is 1.5 mg/kg feed for cattle, salmonids and non-food-producing animals and 1 mg/kg feed for pigs and poultry. For heptan-2-one, 6-methylhept-5-en-2-one, undecan-2-one, octan-2-one, nonan-2-one, octan-3-one, 6-methylhepta-3,5-dien-2-one, tridecan-2-one, nonan-3-one and decan-2-one, the corresponding values are 0.5 mg/kg (cattle, salmonids and non-food-producing animals) and 0.3 mg/kg (pigs and poultry). Mammals, birds and fish share a similar capacity to metabolise secondary alcohols, ketones and esters to innocuous substances. Consequently, no safety concern would arise for consumers from the use of these compounds as proposed in feeds. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considers all the compounds under assessment as irritants to skin, eyes and respiratory tract, and as skin sensitisers. Use of the compounds in animal feed at the maximum safe level is considered safe for the environment, except for nonan-3-one in sea cages, where only 0.047 mg/kg is safe. Since all of the compounds are used in food as flavourings and their function in feed is essentially the same as that in food no demonstration of efficacy is necessary.

Keywords: sensory additives, saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols, chemical group 5, safety

Requestor: European Commission

Question number: EFSA-Q-2010-01040

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), 2015. Scientific opinion on the safety and efficacy of saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols belonging chemical group 5 when used as flavourings for all animal species. EFSA Journal 2015;13(11):4268, 21 pp. doi:10.2903/j.efsa.2015.4268

ISSN: 1831-4732

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

Summary

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 24 compounds (saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols. No aromatic or heteroaromatic moiety as a component of an ester or ketal belonging to chemical group 5) when used as flavourings for all animal species. Because the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) has outstanding concerns about six of the compounds under application when used in food, the FEEDAP Panel will delay its assessment of these compounds until these issues have been resolved. During the assessment, the applicant withdrew the application for 6,10-dimethyl-5,9-undecadien-2-one. The compound has been excluded from further assessment. Consequently this opinion deals with only 17 of the 24 compounds for which application was made.

The use of isopropanol [02.079] and isopropyl tetradecanoate [09.105] in animal feed are safe for all animal species at the proposed maximum dose levels of 25 mg and 5 mg/kg feed, respectively, based on the comparable levels of exposure of both humans and animals to isopropanol. From toxicological data, heptan-2-one [07.002] and pentan-2-one [07.054] are also considered safe at the proposed maximum dose level (5 mg/kg feed) for all species except piglets, chickens for fattening and laying hens. Their calculated safe use level is 4 mg/kg feed for piglets, 3 mg/kg feed for chickens and hens and 2 mg/kg for cats. Since no acceptable toxicological data are available for the remaining compounds, the threshold of toxicological concern approach was used to calculate a maximum safe concentration in feed for each single compound. For octan-2-ol [02.022], pentan-2-ol [02.088], octan-3-ol [02.098] and butan-2-one [07.053], the calculated safe use level is 1.5 mg/kg complete feed for cattle, salmonids and non-food-producing animals and 1 mg/kg complete feed for pigs and poultry. For heptan-2-one [07.002], 6-methylhept-5-en-2-one [07.015], undecan-2-one [07.016], octan-2-one [07.019], nonan-2-one [07.020], octan-3-one [07.062], 6-methylhepta-3,5-dien-2-one [07.099], tridecan-2-one [07.103], nonan-3-one [07.113] and decan-2-one [07.150], the calculated safe use level is 0.5 mg/kg complete feed for cattle, salmonids and non-food-producing animals and 0.3 mg/kg complete feed for pigs and poultry.

Secondary alcohols, ketones and esters with esters containing secondary alcohols are rapidly converted to innocuous substances. Mammals, birds and fish share a similar metabolic capacity to handle these compounds. Consequently, no safety concern would arise for the consumer from the use of these compounds up to the highest safe level in feeds.

In the absence of data, the FEEDAP Panel considers all the compounds under assessment as irritants to skin, eyes and respiratory tract, and also as skin sensitisers.

In the absence of any data, and solely based on modelled predictions of environmental fate and toxicity, the use of nonan-3-one as a feed additive for all animal species at 0.5 mg/kg feed is not expected to pose a risk for the environment, except for sea cages, where only 0.047 mg/kg can be considered safe. The use of the remaining 17 compounds in animal feeding at the maximum safe use level is also considered safe for the environment.

Since all of the compounds under application are used in food as flavourings, and their function in feed is essentially the same as that in food, no demonstration of efficacy is necessary. However, in the absence of data on the proposed dose and 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.

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 received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation of 24 substances belonging to chemical group 5,[3] when used as a feed additive for all animal species (category: sensory additives; functional group: flavourings). Chemical group (CG) 5 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[4] as 'saturated and unsaturated aliphatic secondary alcohol/ketones/esters with esters containing secondary alcohols. No aromatic or heteroaromatic moiety as a component of an ester or ketal'.

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. 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 20 September 2010.

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 on the efficacy of octan-2-ol, isopropanol, pentan-2-ol, octan-3-ol, heptan-2-one, 6-methylhept-5-en-2-one, undecan-2-one, octan-2-one, nonan-2-one, butan-2-one, pentan-2-one, octan-3-one, 6-methylhepta-3,5-dien-2-one, tridecan-2-one, nonan-3-one, decan-2-one, 6,10-dimethyl-5,9-undecadien-2-one and isopropyl tetradecanoate, when used under the proposed conditions of use (see section 3.1.3).

1.2. Additional information

Twenty-one of the 24 compounds have been assessed for safety by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA; WHO, 2000, 2001 and 2002). No Acceptable Daily Intake (ADI) values were specified. According to Regulation (EC) No 1565/2000,[3] 'Substances classified by JECFA as to present no safety concern at the current levels of intake with the exception of substances which have been accepted on the sole basis that their estimated intake is lower than the threshold of concern of 1.5 µg per person per day, as laid down in the reports of the 46th, 49th, 51st and 53rd JECFA meetings need not to be re-evaluated.' For this reason, the 14 substances evaluated by JECFA at the 51st meeting (WHO, 2000) were not evaluated by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) but simply accepted as safe when used as a food flavour.

[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 18 out of the 24 substances under application (see section 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 three compounds under application not yet considered by JECFA are decan-2-one (EU Flavour Information System (FLAVIS) number) [07.216], 6,10-dimethyl-5,9-undecadien-2-one [07.216] and oct-1-en-3-yl acetate [09.281].

The CEF Panel of EFSA supported the conclusions on the safety of the majority of the compounds examined by JECFA (EFSA, 2009a; EFSA CEF Panel 2011, 2012a) but had outstanding concerns about five (EFSA, 2008a, b, 2009b; EFSA CEF Panel 2012b, c). These concerns were triggered by a number of issues, in particular, by a theoretical consideration which suggested the possible genotoxicity of alpha-beta unsaturated ketones. As a result, the CEF Panel made a request for additional *in vivo* genotoxicity tests for 5-methylhept-2-en-4-one [07.139], oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099] and oct-1-en-3-one [07.081] and also for oct-1-en-3-yl acetate [09.281], a compound not evaluated by JECFA (EFSA CEF Panel, 2012b, c). For isopulegol [02.067], the EFSA CEF Panel requested additional toxicity data to complete the assessment (EFSA, 2009b). Because of the concerns raised by the CEF Panel, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) will delay its assessment of these six compounds until the issues have been resolved and conclusions reached about their safe use in food.

EFSA did not assess 6,10-dimethyl-5,9-undecadien-2-one [07.216] but considered its E-isomer (5E)-6,10-dimethyl-5,9-undecadien-2-one [07.123] safe for use as a food flavour (EFSA, 2008a). During the assessment, the applicant withdrew the application for the mixture of the E- and Z-stereoisomers of 6,10-dimethyl-5,9-undecadien-2-one [07.216].

The present opinion concerns only 17 substances, namely octan-2-ol [02.022], isopropanol [02.079], pentan-2-ol [02.088], octan-3-ol [02.098], heptan-2-one [07.022], 6-methylhept-5-en-2-one [07.015], undecan-2-one [07.016], octan-2-one [07.019], nonan-2-one [07.020], butan-2-one [07.053], pentan-2-one [07.054], octan-3-one [07.062], 6-methylhepta-3,5-dien-2-one [07.099], tridecan-2-one [07.103], nonan-3-one [07.113], decan-2-one [07.150], and isopropyl tetradecanoate [09.105].

The 17 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. 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 flavourings from CG 5—saturated and unsaturated aliphatic secondary alcohol/ketones/esters with esters containing secondary alcohols. No aromatic or heteroaromatic

[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-2010-0074.

moiety as a component of an ester or ketal—in animal feed. The Executive Summary of the EURL report can be found in Annex A[8].

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the aliphatic and aromatic hydrocarbons 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 2008c, 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), 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 17 additives under application are shown in Figure 1 and their physico-chemical characteristics in Table 1.

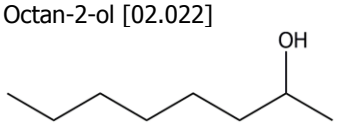
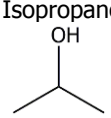
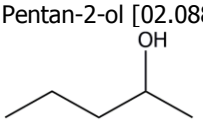
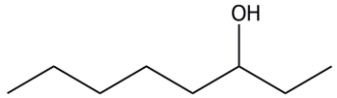
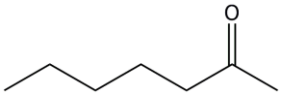
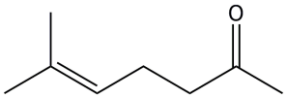
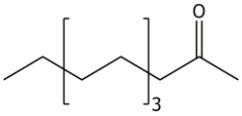
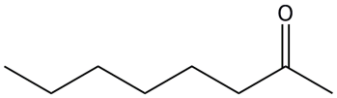
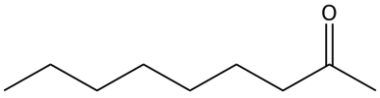
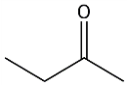
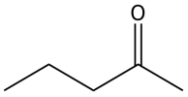
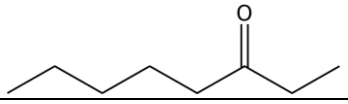
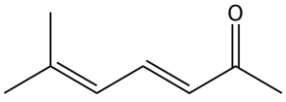
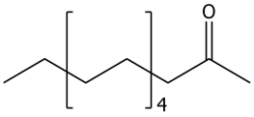
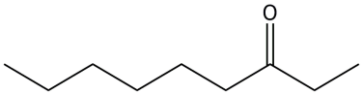
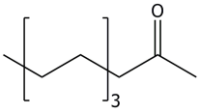
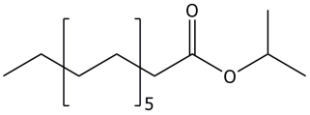
Octan-2-ol [02.022] 	Isopropanol [02.079] 	Pentan-2-ol [02.088] 
Octan-3-ol [02.098] 	Heptan-2-one [07.002] 	6-Methylhept-5-en-2-one [07.015] 
Undecan-2-one [07.016] 	Octan-2-one [07.019] 	Nonan-2-one [07.020] 
Butan-2-one [07.053] 	Pentan-2-one [07.054] 	Octan-3-one [07.062] 
6-Methylhepta-3,5-dien-2-one [07.099] 	Tridecan-2-one [07.103] 	Nonan-3-one [07.113] 
Decan-2-one [07.150] 	Isopropyl tetradecanoate [09.105] 	

Figure 1: Molecular structures and [FLAVIS numbers] of the 17 flavouring compounds under assessment

[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2010-0074?search&form-return

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} (1)
Octan-2-ol	123-96-6	02.022	$C_8H_{18}O$	130.23	Liquid	2.9
Isopropanol	67-63-0	02.079	C_3H_8O	60.1	Liquid	0.05
Pentan-2-ol	6032-29-7	02.088	$C_5H_{12}O$	88.15	Liquid	1.19
Octan-3-ol	589-98-0	02.098	C_6H_7ON	130.23	Liquid	2.73
Heptan-2-one	110-43-0	07.002	$C_5H_{10}O$	114.19	Liquid	1.98
6-Methylhept-5-en-2-one	110-93-0	07.015	$C_8H_{14}O$	126.19	Liquid	2.06
Undecan-2-one	112-12-9	07.016	$C_{11}H_{22}O$	170.3	Liquid	4.09
Octan-2-one	111-13-7	07.019	$C_8H_{16}O$	128.21	Liquid	2.37
Nonan-2-one	821-55-6	07.020	$C_9H_{18}O$	142.24	Liquid	3.14
Butan-2-one	78-93-3	07.053	C_4H_8O	72.11	Liquid	0.29
Pentan-2-one	107-87-9	07.054	$C_5H_{10}O$	86.13	Liquid	0.91
Octan-3-one	106-68-3	07.062	$C_8H_{16}O$	128.21	Liquid	2.22
6-Methylhepta-3,5-dien-2-one	1604-28-0	07.099	$C_8H_{12}O$	124.18	Liquid	1.66
Tridecan-2-one	593-08-8	07.103	$C_{13}H_{26}O$	198.35	Solid	4.68
Nonan-3-one	925-78-0	07.113	$C_9H_{18}O$	142.24	Liquid	2.71
Decan-2-one	693-54-9	07.150	$C_{10}H_{20}O$	156.27	Liquid	3.73
Isopropyl tetradecanoate	110-27-0	09.105	$C_{17}H_{34}O_2$	270.46	Liquid	7.17

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

All of the compounds under consideration are produced by chemical synthesis and typical routes of synthesis are described for each compound.[9]

Data were provided on the batch to batch variation in five batches of each additive except for nonan-3-one [07.113], for which only one batch was available due to the low production volume (< 1 kg/year).[10] The content of the active substance exceeded in all cases the JECFA specifications (Table 2).

Table 2: Identity of the substances and data on purity

EU Register name	FLAVIS No	JECFA specification minimum %(1)	Assay %	
			Average	Range
Octan-2-ol	02.022	97	99.0	98.0–99.8
Isopropanol	02.079	99.7	99.0	99.9–100
Pentan-2-ol	02.088	97.9	99.7	99.7–99.9
Octan-3-ol	02.098	97	98.0	97.2–99.5
Heptan-2-one	07.002	95	99.8	99.6–99.9
6-Methylhept-5-en-2-one	07.015	97	99.3	99.1–99.6
Undecan-2-one	07.016	96	99.7	99.1–100
Octan-2-one	07.019	95	99.7	98.9–99.9
Nonan-2-one	07.020	97	99.7	99.4–99.8
Butan-2-one	07.053	99.5	99.8	99.5–99.9
Pentan-2-one	07.054	95	99.9	99.9–99.9
Octan-3-one	07.062	98	99.4	98.5–99.8
6-Methylhepta-3,5-dien-2-one	07.099	96	99.0	98.7–99.3
Tridecan-2-one	07.103	95	99.0	98.6–99.9
Nonan-3-one	07.113	95.9	98.9(2)	–
Decan-2-one	07.150	98	99.5	99.4–99.7
Isopropyl tetradecanoate	09.105	99	99.5	99.2–99.8

(1): FAO, 2006.
(2): One batch, use of the product is 1 kg/year or less.

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

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

With the exception of octan-3-one [07.062], for which the shelf life is stated to be 6 months, the minimum shelf life of the remaining compounds under assessment ranges from 12 to 60 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.

Although no data are required for the stability of volatile additives in premixes 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 12 out of the 17 compounds in CG 5 have a low water solubility (Log K_{ow} > 2), 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 all of the 17 compounds in feed or water for drinking for all animal species without withdrawal. For isopropanol, the applicant proposes a normal use level of 5 mg/kg feed and a high use level of 25 mg/kg. For the remaining 16 additives, the applicant proposes a normal use level of 1 mg/kg feed and a high use level of 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 high use levels proposed by the applicant (25 mg/kg for isopropanol and 5 mg/kg complete feed for the remaining compounds).

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

Four of the compounds under assessment are saturated aliphatic acyclic secondary alcohols [02.022, 02.079, 02.088 and 02.098], 10 are saturated aliphatic ketones [07.002, 07.016, 07.019, 07.020, 07.053, 07.054, 07.062, 07.103, 07.113 and 07.150], two are unsaturated aliphatic ketones [07.015 and 07.099] and one is an ester of an aliphatic acyclic secondary alcohol and a linear aliphatic carboxylic acid [09.105]. In general, aliphatic secondary alcohols and ketones are expected to be rapidly absorbed in the gastrointestinal tract (WHO, 2000).

The potential metabolic reactions involved in the biotransformation of secondary alcohols, ketones and esters with esters containing secondary alcohols (substances belonging to CG 5) are (i) conjugation of secondary alcohols with glucuronic acid followed by excretion in the urine or bile; (ii) oxidation of secondary alcohols to the corresponding ketone (minor pathway *in vivo* (Kasper and Henton, 1980; WHO, 2000)); (iii) reduction of ketones to the corresponding secondary alcohol with subsequent excretion as conjugate of glucuronic acid; (iv) omega oxidation of short-chain ketones (carbon atoms < 5) or oxidation of the terminal methyl group and subsequent oxidation to yield an alpha-ketocarboxylic acid; (v) oxidation of double bonds; (vi) hydrolysis of esters via carboxylesterases (Heymann, 1980) followed by excretion of the secondary alcohol as glucuronide-conjugate and metabolism of the linear carboxylic acid by beta-oxidation in the fatty acid pathway and citric acid cycle. The expected metabolic reactions in laboratory animals of these substances have been extensively reviewed by the EFSA CEF Panel (EFSA 2009a; EFSA CEF Panel 2012d).

The absorption, distribution, metabolism and elimination (ADME) of isopropanol [02.079] and butan-2-one (methyl ethyl ketone, MEK) [07.053] have been well characterised in mammals. Isopropanol [02.079] is readily absorbed in animals and humans through the lungs, skin and the gastrointestinal tract. It is rapidly distributed throughout the body and has been shown to cross the blood–brain

barrier. Elimination from the blood follows first-order kinetics. Approximately 80 % of an intravenous dose is oxidised to acetone in rats and mice (Slauter et al., 1994). Excretion occurs mainly through the expired air either as unchanged isopropanol or as acetone. Quantities of acetone and isopropanol are excreted in urine together with the glucuronide conjugate of isopropanol. The metabolism of isopropanol is via oxidation by alcohol dehydrogenase (ADH) to acetone. In common with other alpha-substituted (secondary) alcohols, isopropanol is a relatively poor substrate for ADH. The primary metabolite, acetone, is eliminated in the expired air and in the urine and also undergoes further oxidation to acetate, formate and ultimately CO_2 (IARC, 1999).

Absorption of butan-2-one [07.053] is rapid via inhalation and ingestion. Butan-2-one is a small water-soluble uncharged non-polar substance. Therefore, after absorption it is expected to distribute uniformly to the various soft tissue compartments but it is not expected to accumulate (Perbellini et al., 1984; Lowry, 1987, as cited in ATSDR, 1992). It can cross the placenta (Dowty et al., 1976, as cited in IPCS, 1993). Butan-2-one occurs naturally in the human body as a metabolite of alpha-methylacetoacetic acid (Browning, 1965, as cited in ATSDR, 1992), as a product of isoleucine catabolism (Tsao and Pfeiffer, 1957, as cited in IPCS, 1993; Przyrembel et al., 1979). It can follow two main metabolic pathways, one reductive or the other oxidative (Brady et al., 1989, as cited in US-EPA, 2003d; Traiger et al., 1989, as cited in ATSDR, 1992). All butan-2-one metabolites can be further metabolised to CO_2 (Liira et al., 1988) or converted to O-glucuronides and O-sulphates before elimination (DiVincenzo et al., 1976, as cited in US-EPA, 2003d).

Studies of metabolism of compounds belonging to CG 5 in animals, other than rats, are lacking in the scientific literature. In mammals, oxidation is ubiquitous and phase II conjugation via glucuronidation, sulphation or addition of glycine occur in mammals, although the predominance of one pathway over another varies among animal species (Gupta, 2007). Data collected in a review by Ioannides (2006) show that the cytochrome P450 enzymes responsible for the majority of oxidation reactions are expressed in the liver of the main food-producing animals (cattle, pig, sheep, goat) as well as in the rabbit and chicken (Nebbia et al., 2003). Reductases to reduce carbonyl groups in xenobiotics were also found in farm animals, namely cattle, pig, sheep and goat (Szotáková et al., 2004). Biotransformation through oxidation followed by conjugation with glucuronic acid, sulphate and glycine has also been reported for birds (Pan and Fouts, 1978). A recent study showed that the principal cytochrome P450 enzymes responsible for oxidation of xenobiotics, as well as glutathione transferases, are present in the liver of chickens (Blevins et al., 2012). Carboxylesterase activity also plays a significant role in detoxification processes in birds (Beasley, 1999) as well as in fish (Di Giulio and Hinton, 2008; Tocher, 2003). Fish have analogous mechanisms for handling xenobiotic compounds, including both phase 1 and phase 2 biotransformation reactions, and many of the same microsomal and cytosolic enzymes as mammals (Wolf and Wolfe, 2005). Thus, fish can transform endobiotic and xenobiotic compounds through oxidation or hydroxylation, conjugate the metabolites to polar substrates through sulphate, glucuronide, glutathione and amino acids conjugation (James and Pritchard, 1987) with further elimination via bile or urine (Di Giulio and Hinton, 2008). Therefore, food-producing animals, including fish and birds, can also be assumed to have the ability to metabolise and excrete the flavouring substances from CG 5 and there is no evidence that they or their metabolites would accumulate in tissues and cause a concern for consumer safety.

3.2.2. Toxicological studies

Toxicological data (subchronic, repeated-dose studies, with multiple doses tested) could be found for heptan-2-one [07.002] and pentan-2-one [07.054].

For heptan-2-one [07.002], a No Observed Adverse Effect Level (NOAEL) of 20 mg/kg body weight (bw) per day was identified in a 13-week study in rats (15 males, 15 females; administration route: oral gavage; doses: 0, 20, 100 and 500 mg/kg bw per day). No effects were seen on food and water consumption, body weight gain, haematological parameters and histopathology. A dose-related increase in relative kidney weight and increased excretion of cells in the urine was observed at 100 and 500 mg/kg bw per day in male rats. Although these effects were not accompanied by abnormal renal function or histopathological damage, they were considered adverse, since excretion of cells has been suggested as a sensitive test of proximal renal tubular damage (Gaunt et al., 1972).

A 13-week study in rats made with three graded doses of pentan-2-one [07.054] supplied via drinking water was also identified (O'Donohogue et al., 1978, unpublished).[11] In this study the only adverse effect reported was a reduction in growth of approximately 9 % seen with the highest dose applied (1.0 % calculated to be equivalent to 450 mg/kg bw). Otherwise clinical signs, organ weights and histology findings were all reported to be normal. From this study a No Observed Effect Level (NOEL) of 259 mg/kg bw was derived. However, although this study has been extensively described in secondary sources, the original study report is no longer available.

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 0.1 to 84 000 µg/person per day (EFSA, 2008a, 2009b). This corresponds to 0.005 to 3 896 µg/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 25 mg/kg feed for isopropanol and 5 mg/kg feed for the others) 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$^{0.75}$/day		
			Salmon	Piglet	Dairy cow
Octan-2-ol	5	0.51	118	526	777
Isopropanol	25	3 896	588	2 632	3 885
Pentan-2-ol	5	0.28(2)	118	526	777
Octan-3-ol	5	0.22	118	526	777
Heptan-2-one	5	4.45	118	526	777
6-Methylhept-5-en-2-one	5	4.64	118	526	777
Undecan-2-one	5	15.31	118	526	777
Octan-2-one	5	4.31	118	526	777
Nonan-2-one	5	14.84	118	526	777
Butan-2-one	5	4.45	118	526	777
Pentan-2-one	5	5.57	118	526	777
Octan-3-one	5	0.13	118	526	777
6-Methylhepta-3,5-dien-2-one	5	0.70(2)	118	526	777
Tridecan-2-one	5	2.88	118	526	777
Nonan-3-one	5	0.005(2)	118	526	777
Decan-2-one	5	0.02	118	526	777
Isopropyl tetradecanoate	5	0.88	118	526	777

(1): Metabolic body weight (kg bw$^{0.75}$) for a 60-kg person = 21.6.
(2): Exposure based on EU intake as reported by JECFA (WHO, 2000).

Table 3 shows that for all compounds, except isopropanol [02.079], the intake by the 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 for these compounds cannot be derived from the risk assessment for food use. For isopropanol the proposed highest animal exposure is similar to human exposure. The FEEDAP Panel concludes that isopropanol is safe for the target species at the proposed maximum dose level (25 mg/kg feed). This conclusion is also extended to isopropyl tetradecanoate [09.105], which is considered safe at the maximum proposed dose of 5 mg/kg complete feed. This compound is fully and rapidly hydrolysed to the alcohol and to a saturated fatty acid forming a normal part of diet.

Toxicological data (subchronic, repeated-dose studies) were available for only heptan-2-one [07.002], from which a NOAEL value could be derived (see section 3.2.2). For pentan-2-one [07.054], a chronic study was found but the original study report was not available. Considering the chemical similarity

[11] Technical Dossier/Supplementary inforamtion May 2012/Annex_literature_CDG05, pp139, O'Donohogue JL, Krasavage WJ and Terhaar CJ, 1978. A comparative chronic toxicity study of methyl propyl ketone, methyl n-butyl ketone, and hexane by ingestion. Private communication to FEMA.

between heptan-2-one and pentan-2-one, the FEEDAP Panel applies the more conservative NOAEL of 20 mg/kg bw to both compounds. Applying an uncertainty factor (UF) of 100 to the NOAEL, the maximum safe intake for the target species was derived for the compounds following the EFSA Guidance for sensory additives (EFSA FEEDAP Panel, 2012a), and thus the maximum safe feed concentration was calculated (Table 4).

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 3.2.1), their use as additives in cat feed needs an additional UF 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 heptan-2-one [07.002] and pentan-2-one [07.054]

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	0.4	10
Veal calves (milk replacer)	100	2 000	20	10
Cattle for fattening	400	8 000	80	9
Pigs for fattening	100	3 000	20	7
Sows	200	6 000	40	7
Dairy cows	650	20 000	130	6
Turkeys for fattening	12	400	2.4	6
Piglets	20	1 000	4.0	4
Chickens for fattening	2	120	0.4	3
Laying hens	2	120	0.4	3
Dogs	15	250	3.0	11
Cats	3	60	0.6	2(3)

(1): 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.
(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.

For the 13 remaining compounds, subchronic, repeated-dose studies performed with the additive under assessment were not available (12 compounds) or were submitted only as a summary report (6-methylhept-5-en-2-one). Therefore, the threshold of toxicological concern (TTC) approach, currently applied to estimate the acceptable exposure level for humans, was followed to derive the maximum safe feed concentration (EFSA FEEDAP Panel, 2012a).

For Cramer Class I compounds, i.e. octan-2-ol [02.022], pentan-2-ol [02.088], octan-3-ol [02.098] and butan-2-one [07.053], the calculated safe use level is 1.5 mg/kg complete feed for cattle, salmonids and non-food producing animals and 1 mg/kg complete feed for pigs and poultry.

For Cramer Class II compounds, i.e. 6-methylhept-5-en-2-one [07.015], undecan-2-one [07.016], octan-2-one [07.019], nonan-2-one [07.020], octan-3-one [07.062], 6-methylhepta-3,5-dien-2-one [07.099], tridecan-2-one [07.103], nonan-3-one [07.113] and decan-2-one [07.150] the calculated safe use level is 0.5 mg/kg complete feed for cattle, salmonids and non-food producing animals and 0.3 mg/kg complete feed for pigs and poultry.

Conclusions on safety for the target species

The FEEDAP Panel concludes that:

- isopropanol [02.079] is safe for the target species at the proposed maximum dose level (25 mg/kg feed), as the human exposure exceeds or is similar to that of the proposed animal exposure. This conclusion is extended to include isopropyl tetradecanoate [09.105] which is considered safe at the maximum proposed dose of 5 mg/kg complete feed;
- heptan-2-one [07.002] and pentan-2-one [07.054] are safe at the proposed maximum dose level (5 mg/kg feed) for all species, except piglets, chickens for fattening, laying hens and

cats. The calculated safe use level is 4 mg/kg feed for piglets, 3 mg/kg feed for chickens and hens and 2 mg/kg feed for cats;

- for the four compounds belonging to Cramer Class I, octan-2-ol [02.022], pentan-2-ol [02.088], octan-3-ol [02.098] and butan-2-one [07.053], the calculated safe use level is 1.5 mg/kg complete feed for cattle, salmonids and non-food producing animals and 1 mg/kg complete feed for pigs and poultry;
- for the nine compounds belonging to Cramer Class II, 6-methylhept-5-en-2-one [07.015], undecan-2-one [07.016], octan-2-one [07.019], nonan-2-one [07.020], octan-3-one [07.062], 6-methylhepta-3,5-dien-2-one [07.099], tridecan-2-one [07.103], nonan-3-one [07.113] and decan-2-one [07.150], the calculated safe use level is 0.5 mg/kg complete feed for cattle, salmonids and non-food producing animals and 0.3 mg/kg complete feed for pigs and poultry.

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, 2000, 2001, 2002) and EFSA (EFSA, 2009b; EFSA CEF Panel, 2011, 2012a, b, c). No ADI values were established. All compounds are currently authorised in the EU as food additives without limitations.[12]

The compounds under assessment in CG 5 are secondary alcohols, ketones and esters with esters containing secondary alcohols which are rapidly converted to innocuous substances. Mammals, birds and fish share a similar metabolic capacity to handle these compounds. Consequently, no safety concern would arise for the consumer from the use of these compounds up to the highest safe level in feeds.

3.2.5. Safety for the user

No specific data on the safety for the user were provided. In the material safety data sheets[13] hazards for skin and eye contact and respiratory exposure are recognised for the majority of the compounds under application. Most of them are classified as irritating to the respiratory system.

In the absence of data, the FEEDAP Panel considers all the compounds under assessment as irritants to skin, eyes and respiratory tract, and also as skin sensitisers.

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 13 of the substances under assessment, namely octan-2-ol [02.022], pentan-2-ol [02.088], octan-3-ol [02.098], heptan-2-one [07.022], 6-methylhept-5-en-2-one [07.015], undecan-2-one [07.016], octan-2-one [07.019], nonan-2-one [07.020], pentan-2-one [07.054], octan-3-one [07.062], 6-methylhepta-3,5-dien-2-one [07.099], tridecan-2-one [07.103] and decan-2-one [07.150] (Data taken from the Netherlands Organisation for Applied Scientific Research (TNO) database Volatile Compounds in Food *ver.* 14.1; Burdock, 2003).[14]

Isopropanol [02.079], butan-2-one [07.053] and isopropyl tetradecanoate [09.105] do not occur in the environment at levels above the application rate of 25 and 5 mg/kg feed. However, these compounds are expected to be metabolised by the target species and excreted as innocuous compounds (see section 3.2.1). Therefore no environmental risk is foreseen for these compounds.

Although nonan-3-one [07.113] occurs naturally, data on its distribution and quantification are limited. Therefore, a further environmental risk assessment was performed based on a dose of 0.5 mg/kg feed, which is the safe level for the target species for a Cramer Class II compound. When the predicted environmental concentration (PEC) for soil (PEC_{soil}) are calculated according to the EFSA

[12] 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.
[13] Technical dossier/Section II/Annex II.3.
[14] Technical dossier/ Supplementary information June 2011.

guidance (EFSA, 2008c) with a fixed concentration in feed, there is a fixed order of PEC_{soil} from each species, with the lamb being the most critical. The use of fish feed in sea cages can result in a PEC for sediment above 10 µg/kg when the concentration in fish feed is above 0.047 mg/kg, regardless of the properties of the additive, when calculated according to the guidance (EFSA, 2008c).

The application of 0.5 mg additive/kg feed might cause a maximum predicted soil concentration of 11 µg/kg soil dry weight (dw), which is slightly above the threshold of 10 µg/kg (EFSA, 2008c). The PEC for pore water, however, is dependent on the sorption, which is different for each compound. For these calculations, the substance-dependent constants K_{oc} (organic carbon sorption constant), molecular weight, vapour pressure and solubility are needed. These were estimated from the SMILES (Simplified Molecular Input Line Entry Specification) notation of the chemical structure using EPIWEB 4.1 (Table 5).[15] 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.

Table 5: Physico-chemical properties predicted by EPIWEB 4.1 and predicted toxicity of nonan-3-one [07.113] by ECOSAR 1.11

Predicted by EPIWEB 4.1					Predicted by ECOSAR 1.11			
DT_{50} (1)	Molecular weight	Vapour pressure	Solubility	K_{oc} (2)	LC_{50} (3) fish	LC_{50} *Daphnia*	EC_{50} (4) algae	EC_{50} earthworm
(days)	(g/mol)	(Pa)	(mg/L)	(L/kg)	(mg/L)	(mg/L)	(mg/L)	(mg/kg)
5	142.24	111	396	95	26.825	16.266	15.899	208.66

(1): DT_{50}, half-life of the additive in manure.
(2): K_{oc}, organic carbon sorption constant.
(3): LC_{50}, the concentration of a test substance which results in a 50 % mortality of the test species.
(4): 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).

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 of 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.

A groundwater concentration of 6.0 µg/L was estimated, which is above the threshold of 0.1 µg/L, indicating the need for a phase II environmental 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}) for earthworms, fish, algae and *Daphnia* from the SMILES notation of the substance (Table 5).

The EC_{50} of earthworm is 208.66 mg/kg. This would yield a Predicted No Effect Concentration (PNEC) of 208.66 µg/kg, which is higher than the estimated soil concentration (11 µg/kg). Thus, in absence of experimental data, modelling of PEC and PNEC indicates that there is probably no risk for the soil compartment. The lowest estimated EC_{50} value is 15.899 mg/L for algae, as indicated above. This would yield a PNEC of 15.899 µg/L, using an uncertainty factor of 1 000 according to the guidance document (EFSA, 2008c), which is higher than the estimated surface water concentration (2.0 µg/L).

The use nonan-3-one [07.113] in fish feed in aquaculture does not give a PEC above the trigger value of 0.1 µg/L when calculated according to the guidance. For sea cages a safe dose of 0.047 mg/kg feed was calculated according to the EFSA guidance (EFSA, 2008c). This dose would give a sediment concentration of 10 µg/kg, which is the threshold level of no concern.

Conclusions on the safety for the environment

In the absence of any data and solely based on modelled predictions of environmental fate and toxicity, the use of nonan-3-one as a feed additive for all animal species at 0.5 mg/kg feed is not expected to pose a risk for the environment, except for sea cages, where only 0.047 mg/kg can be

[15] Available online: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm

considered safe. All the other compounds under assessment are safe for the environment at the use level considered safe for the target species.

3.3. Efficacy

Since all 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 use of isopropanol [02.079] and isopropyl tetradecanoate [09.105] in animal feed are safe for all animal species at the proposed maximum dose levels of 25 mg and 5 mg/kg feed, respectively. Heptan-2-one [07.002] and pentan-2-one [07.054] are also safe at the proposed maximum dose level (5 mg/kg feed) for all species, except piglets, chickens for fattening and laying hens. Their calculated safe use level is 4 mg/kg feed for piglets, 3 mg/kg feed for chickens and hens and 2 mg/kg for cats. For octan-2-ol [02.022], pentan-2-ol [02.088], octan-3-ol [02.098] and butan-2-one [07.053], the calculated safe use level is 1.5 mg/kg complete feed for cattle, salmonids and non-food-producing animals and 1 mg/kg complete feed for pigs and poultry. For 6-methylhept-5-en-2-one [07.015], undecan-2-one [07.016], octan-2-one [07.019], nonan-2-one [07.020], octan-3-one [07.062], 6-methylhepta-3,5-dien-2-one [07.099], tridecan-2-one [07.103], nonan-3-one [07.113] and decan-2-one [07.150], the calculated safe use level is 0.5 mg/kg complete feed for cattle, salmonids and non-food-producing animals and 0.3 mg/kg complete feed for pigs and poultry.

Secondary alcohols, ketones and esters with esters containing secondary alcohols are rapidly converted to innocuous substances. Mammals, birds and fish share a similar metabolic capacity to handle these compounds. Consequently, no safety concern would arise for the consumer from the use of these compounds up to the highest safe level in feeds.

In the absence of data, the FEEDAP Panel considers all the compounds under assessment as irritants to skin, eyes and respiratory tract, and also as skin sensitisers.

In the absence of any data and solely based on modelled predictions of environmental fate and toxicity, the use of nonan-3-one as a feed additive for all animal species at 0.5 mg/kg feed is not expected to pose a risk for the environment except for sea cages, where only 0.047 mg/kg can be considered safe. Use of the remaining 16 compounds in animal feed at their maximum safe use levels is also considered safe for the environment.

Since 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 proposed dose and 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.

Documentation provided to EFSA

1. Chemically defined flavourings from Flavouring Group 5—Saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols. No aromatic or heteroaromatic moiety as a component of an ester or ketal (CDG 05). August 2010. Submitted by FEFANA Asbl/Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

2. Chemically defined flavourings from Flavouring Group 5—Saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols. No aromatic or heteroaromatic moiety as a component of an ester or ketal (CDG 05). June 2011. Submitted by FEFANA Asbl/Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

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Abbreviations

ADI	Acceptable Daily Intake
bw	body weight
$bw^{0.75}$	metabolic body weight
CAS	Chemical Abstracts Service
CDG	chemically defined group
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
DM	dry matter
DT_{50}	half-life of additive in manure
EC	European Commission
EC_{50}	half-maximal effective concentration
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FAO	Food Agriculture Organization of the United Nations
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
K_{oc}	organic carbon sorption constant
Log K_{ow}	logarithm of octanol–water partition coefficient
MEK	methyl ethyl ketone
NOAEL	No Observed Adverse Effect Level
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
SMILES	Simplified Molecular Input Line Entry Specification
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 Saturated and unsaturated aliphatic secondary alcohols/ketones/ketals/esters with esters containing secondary alcohols. No aromatic or heteroaromatic moiety as component of an ester or ketal

The *Chemically Defined Flavourings – Group 05 (CDG05, Saturated and unsaturated aliphatic secondary alcohols/ketones/ketals/esters with esters containing secondary alcohols. No aromatic or heteroaromatic moiety as component of an ester or ketal)*, in this application comprises twenty-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 95 % to 99.5%.

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 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 *CDG05* 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 CDG05 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 CDG05 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.