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Safety and efficacy of secondary aliphatic saturated or unsaturated alcohols, ketones, ketals and esters with a second secondary or tertiary oxygenated functional group belonging to chemical group 10 when used as flavourings for all animal species

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of 11 compounds belonging to chemical group 10. They are currently authorised as flavours in food. The FEEDAP Panel concludes that: diacetyl [07.052] is safe at the proposed maximum use level of 25 mg/kg complete feed for all target species, except piglets, chickens for fattening, laying hens and cats, for which the proposed normal use level of 5 mg/kg is safe; 3-hydroxybutan-2-one [07.051], 3-methylcyclo-pentan-1,2-dione [07.056], 3-ethylcyclopentan-1,2-dione [07.057], pentan-2,3-dione [07.060], 3,4-dimethylcyclopentan-1,2-dione [07.075], 3,5-dimethyl cyclopentan-1,2-dione [07.076], hexan-3,4-dione [07.077] and sec-butan-3-onyl acetate [09.186] are safe at the proposed maximum dose level of 5 mg/kg for all target species; 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109] and 3-methylnona-2,4-dione [07.184] are safe only at concentrations below the proposed use levels (0.5 mg/kg for cattle, salmonids and non-food producing animals, and 0.3 mg/kg for pigs and poultry). No safety concern would arise for the consumer from the use of these compounds up to the highest proposed level in feeds. Hazards for skin and eye contact and respiratory exposure are recognised for the majority of the compounds under application. Most are classified as irritating to the respiratory system. For 3-hydroxybutan-2-one [07.051], diacetyl [07.052], pentan-2,3-dione [07.060], hexan-3,4-dione [07.077], 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109], 3-methylnona-2,4-dione [07.184] and sec-butan-3-onyl acetate [09.186], the maximum proposed use levels are considered safe for the environment. For cyclopentanediones (3-methylcyclopentan-1,2-dione [07.056], 3-ethylcyclopentan-1.2-dione [07.057], 3,4 dimethylcyclopentan-1,2-dione [07.075] and 3,5-dimethylcyclopentan-1,2-dione [07.076]) usage at levels up to 0.5 mg/kg feed is unlikely to have an adverse effect on the terrestrial or freshwater environments. Because all the compounds under assessment are used in food as flavourings and their function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

Keywords: sensory additives, aromatic ketones, secondary alcohols, related esters, safety, chemical group 10

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Table of contents

Abstract 1
1. Introduction 4
1.1. Background and Terms of Reference 5
1.2. Additional information 4
2. Data and methodologies 5
2.1. Data 5
2.2. Methodologies 5
3. Assessment 5
3.1. Characterisation 5
3.1.1. Characterisation of the flavouring additives 5
3.1.2. Stability 7
3.1.3. Conditions of use 7
3.2. Safety 8
3.2.1. Absorption, distribution, metabolism and excretion (ADME) 8
3.2.2. Toxicological studies 9
3.2.3. Safety for the target species 10
3.2.3.1. Conclusions on safety for the target species 12
3.2.4. Safety for the consumer 12
3.2.5. Safety for the user 12
3.2.6. Safety for the environment 12
3.2.6.1. Conclusions on safety for the environment 14
3.3. Efficacy 14
4. Conclusions 15
Documentation provided to EFSA 15
References 16
Abbreviations 18
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group 19

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7 and in addition, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of 7 years after the entry into force of this Regulation.

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation of 11 substances belonging to chemical group (CG) 10 (3-hydroxybutan-2-one, diacetyl, 3-methylcyclo-pentan-1,2-dione, 3-ethylcyclopentan-1,2-dione, pentan-2,3-dione, 3,4-dimethylcyclopentan-1,2-dione, 3,5-dimethylcyclopentan-1,2-dione, hexan-3,4-dione, 2,6,6-trimethylcyclohex-2-en-1,4-dione, 3-methylnona-2,4-dione and sec-butan-3-onyl acetate), when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). CG 10 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[3] as 'secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group'.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). During the course of the assessment, the applicant withdrew the application for the use of chemically defined flavourings in water for drinking.[4] EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 1 July 2010.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment, and on the efficacy of 3-hydroxybutan-2-one [The EU Flavour Information System (FLAVIS) Number 07.051], diacetyl [07.052], 3-methylcyclopentan-1,2-dione [07.056], 3-ethylcyclopentan-1,2-dione [07.057], pentan-2,3-dione [07.060], 3,4-dimethylcyclopentan-1, 2-dione [07.075], 3,5-dimethylcyclopentan-1,2-dione [07.076], hexan-3,4-dione [07.077], 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109], 3-methylnona-2,4-dione [07.184] and sec-butan-3-onyl acetate [09.186], when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

Nine of the 11 substances have been assessed by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA; WHO, 2000) and were considered safe for use in food. No acceptable daily intake (ADI) values were established. The two compounds not assessed were 2,6,6-trimethyl-cyclohex-2-en-1,4-dione [07.109] and 3-methylnona-2,4-dione [07.184].

Subsequently, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) assessed the compounds belonging to CG 10 and concluded that none of the 11 compounds under application gave rise to safety concerns when used as flavour in food (EFSA 2008a, 2009; EFSA CEF Panel, 2011, 2014a,b,c).

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

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

All 11 compounds are all currently listed in the European Union database of flavouring substances[5] and in the European Union Register of Feed Additives, and thus authorised for use in food and feed in the European Union (EU), respectively. They have not been previously assessed by EFSA as feed additives.

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

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[7] in support of the authorisation request for the use of the compounds belonging to CG 10 as feed additives. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008 and the applicable EFSA guidance documents.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 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 of the 'secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group' 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 aromatic ketones, secondary alcohols and related esters 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, 2008b), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

3.1. Characterisation

3.1.1. Characterisation of the flavouring additives

The molecular structures of the 11 additives under application are shown in Figure 1 and their physicochemical characteristics in Table 1.

[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-2010-0026.

[8] The full report is available on the EURL website https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0026.pdf

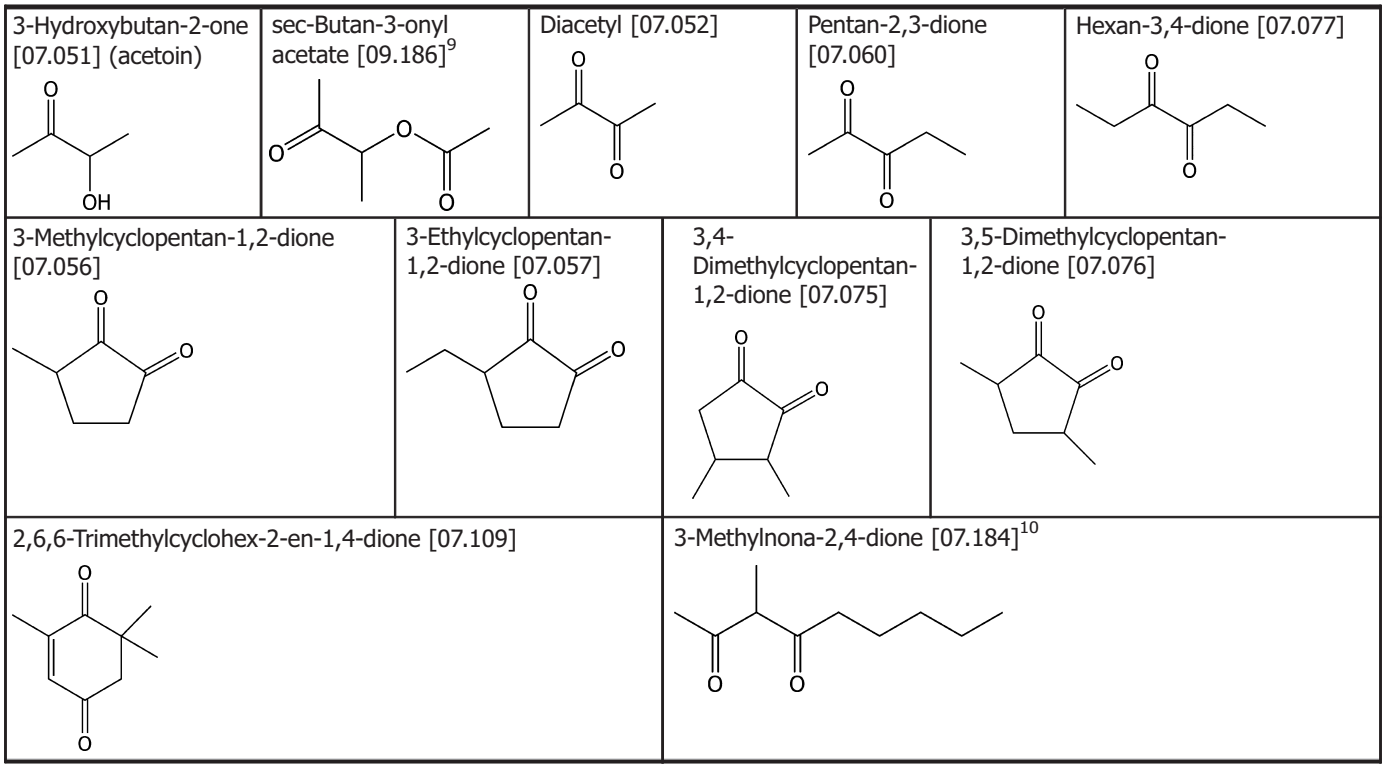

Figure 1: Molecular structures and [FLAVIS numbers] of the 11 flavouring compounds under assessment

The stereochemical configuration is not taken into account in the structures shown in Figure 1 due to various isomers resulting from the keto-enolic tautomerism of the majority of the compounds.

Table 1: Chemical Abstracts Service (CAS) and FLAVIS numbers and some characteristics of the 11 flavouring compounds under assessment

EU register name	CAS No	FLAVIS no	Molecular formula	Molecular weight	Physical state	Log K_{ow} (1)
3-Hydroxybutan-2-one	513-86-0	07.051	$C_4H_8O_2$	88.11	Liquid	−0.36
Diacetyl	431-03-8	07.052	$C_4H_6O_2$	86.09	Liquid	−1.34
3-Methylcyclopentan-1,2-dione	80-71-7	07.056	$C_6H_8O_2$	112.13	Solid	0.30
3-Ethylcyclopentan-1,2-dione	21835-01-8	07.057	$C_7H_{10}O_2$	126.16	Solid	0.83
Pentan-2,3-dione	600-14-6	07.060	$C_5H_8O_2$	100.12	Liquid	−0.85
3,4-Dimethylcyclopentan-1,2-dione	13494-06-9	07.075	$C_7H_{10}O_2$	126.12	Solid	0.53
3,5-Dimethylcyclopentan-1,2-dione	13494-07-0	07.076	$C_7H_{10}O_2$	126.12	Solid	0.53
Hexan-3,4-dione	4437-51-8	07.077	$C_6H_{10}O_2$	114.14	Liquid	−0.35
2,6,6-Trimethylcyclohex-2-en-1,4-dione	1125-21-9	07.109	$C_9H_{12}O_2$	152.2	Solid	0.76
3-Methylnona-2,4-dione	113486-29-6	07.184	$C_{10}H_{18}O_2$	170.25	Liquid	2.81
sec-Butan-3-onyl acetate	4906-24-5	09.186	$C_6H_{12}O_3$	130.14	Liquid	0.68

EU: European Union; FLAVIS number: EU Flavour Information System numbers.
(1): Logarithm of octanol–water partition coefficient.

[9] (R)- or (S)- isomer not specified.
[10] Racemate.

These substances are produced by chemical synthesis. Routes of synthesis are described in the dossier only for five of the 11 compounds under assessment. However, all six of the compounds for which routes of synthesis were not made available, show a high degree of purity.[11]

Batch-to-batch variation data were provided for five batches of each additive except 3,5-dimethylcyclopentan-1,2-dione [07.076], 3-methylnona-2,4-dione [07.184] and sec-butan-3-onyl acetate [09.186] for which only one, three and four batches, respectively, were provided owing to the low use volume.[12] The content of the active substance for all compounds exceeded the JECFA specifications (Table 2), except for 3-ethylcyclopentan-1,2-dione [07.057]. Data for this compound is provided only for a formulated product which contains 40% propylene glycol by weight. Propylene glycol is a recognised food additive (E 1520) and is used as a solvent/carrier for flavourings.[13] Both JECFA (WHO, 1974) and the Scientific Committee on Food (SCF; European Commission, 1978, 1986) assessed propylene glycol. An ADI of 25 mg/kg body weight (bw) per day was established by JECFA and considered acceptable by the SCF for the use of propylene glycol in materials in contact with food (EC, 1986).

Table 2: Identity of the substances and data on purity

EU register name	FLAVIS no	JECFA specification minimum %(1)	Assay %	
			Average	Range
3-Hydroxybutan-2-one	07.051	> 96	98.6	97.0–100
Diacetyl	07.052	> 95	99.6	98.8–99.8
3-Methylcyclopentan-1,2-dione	07.056	> 95	99.9	99.7–100
3-Ethylcyclopentan-1,2-dione	07.057	> 90	62.0(2)	61.5–62.5
Pentan-2,3-dione	07.060	> 93	98.6	98.5–99.1
3,4-Dimethylcyclopentan-1,2-dione	07.075	> 98	99.8	99.7–100
3,5-Dimethylcyclopentan-1,2-dione	07.076	> 97	97.8(3)	97.8
Hexan-3,4-dione	07.077	> 97	97.7	97.2–98.3
2,6,6-Trimethylcyclohex-2-en-1,4-dione	07.109	> 98	99.0	99.0–99.3
3-Methylnona-2,4-dione	07.184	> 97	97.7(4)	97.2–98.3
sec-Butan-3-onyl acetate	09.186	> 98	99.8(5)	99.2–100

FLAVIS number: EU Flavour Information System numbers; JECFA: The Joint FAO/WHO Expert Committee on Food Additives.
(1): FAO, 2006.
(2): The product is diluted in propylene glycol 40%.
(3): One batch, use of the product 1 kg/year or less.
(4): Three batches only, use of the product 1 kg/a or less.
(5): Four batches only, use of the product 1 kg/a or less.

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

3.1.2. Stability

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

3.1.3. Conditions of use

The applicant proposes the use of all of the 11 additives in feed for all animal species without withdrawal. For diacetyl [07.052], the applicant proposes a normal use level of 5 mg/kg feed and a high use level of 25 mg/kg. For the remaining 10 additives, the applicant proposes a normal use level of 1 mg/kg feed and a high use level of 5 mg/kg.

[11] Technical dossier/Section II.
[12] Technical dossier/Section II/Annex 2.1 and Supplementary information May 2011.
[13] Commission Regulation (EU) No 1130/2011 of the European Parliament and of the Council of 11 November 2011 amending Annex III to Regulation (EC) No 1338/2008 of the European Parliament and of the Council on food additives establishing a Union list of food additives approved for use in food additives, food enzymes, food flavourings and nutrients. OJ L 295, 12.11.2011, p. 178.

3.2. Safety

The assessment of safety is based on the highest use level proposed by the applicant (25 mg/kg complete feed for diacetyl and 5 mg/kg complete feed for the remaining compounds).

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

Compounds belonging to CG 10 are absorbed from the gastrointestinal tract (Gabriel et al., 1972) and share common pathways of metabolism: (i) hydrolysis of esters by carboxylesterases, (ii) reduction of ketones to alcohols, (iii) oxidation of alcohols to acids, (iv) α–hydroxylation of the terminal methyl group to yield corresponding ketocarboxylic acids, (v) oxidative decarboxylation to yield carbon dioxide and an aliphatic carboxylic acid, and (vi) conjugation of α-hydroxyketones or their diol metabolites with glucuronic acid (WHO, 1999, 2000; EFSA CEF Panel, 2014c).

Aliphatic acyclic diketones [07.052, 07.060 and 07.077] and α–hydroxyketones [07.051], which contain a carbonyl function at the 2-position (i.e. a methyl ketone) are expected to undergo α–hydroxylation and subsequent oxidation of the terminal methyl group to eventually yield corresponding ketocarboxylic acids. These compounds are intermediary metabolites (e.g. α-ketoacids), which may undergo oxidative decarboxylation to yield carbon dioxide and an aliphatic carboxylic acid. The acid is then metabolised via β-oxidation and the citric acid cycle. β-Ketoacids and derivatives readily undergo decarboxylation to yield breakdown products, which are incorporated into normal biochemical pathways (EFSA, 2008a). Alternatively, the methyl-substituted diketones may be successively reduced to the corresponding hydroxyketones and diols, which are excreted in the urine as glucuronic acid conjugates. This pathway is favoured at elevated *in vivo* concentrations, especially for longer chain length ketones. If the carbonyl function is located elsewhere on the chain, reduction is the predominant pathway. α-Hydroxyketones or their diol metabolites may be excreted as glucuronic acid conjugates (WHO, 1999).

Low concentrations of aliphatic acyclic methyl ketones are mainly metabolised by oxidation of the terminal methyl group. At higher concentrations, acyclic α-diketones are metabolised via a reduction pathway to the diol and subsequent conjugation with glucuronic acid (WHO, 1999; EFSA CEF Panel, 2014b; FGE.09Rev5).

In rats and mice, orally administered acetoin (3-hydroxybutan-2-one [07.051]) is rapidly absorbed from the gastrointestinal tract (Gabriel et al., 1972). Upon intraperitoneal injection of acetoin-2,3-^{14}C to albino rats, $^{14}CO_2$ (representing 15% of the original dose) appeared in the expired air. Acetoin is metabolised primarily via oxidation at low concentrations *in vivo* and by reduction to 2,3-butanediol (butane-2,3-diol) at high concentrations. It is estimated that the rat liver is capable of oxidising 86 μg (1 μmol) acetoin/g liver per day (Gabriel et al., 1972).

Otsuka et al., 1996, demonstrated the high activities of diacetyl- and acetoin-reducing enzymes, in homogenate tissues of rats, especially in the liver, but also in the kidney and brain. One hour after oral administration of diacetyl to rats the amount of the compound in the liver, kidney and brain was 0.03%. Diacetyl was reduced to acetoin, which was mainly present in the brain. 2,3-Butanediol was also present in the three organs, amounting to about 2.3% of the administered dose. When acetoin was orally administered, it was also interconverted into diacetyl and 2,3-butanediol, being mainly present in the brain.

Diacetyl and acetoin are reported to be formed endogenously in humans and cats when pyruvate is converted to diacetyl and acetoin by pyruvate decarboxylase (Gabriel et al., 1972).

The major metabolic pathway for cyclopentanones was demonstrated in rabbits to involve the reduction of the ketone to the corresponding secondary alcohol followed by conjugation of the alcohol with glucuronic acid (Belsito et al., 2012). After oral gavage of cyclopentanone (193 mg/kg body weight), approximately half of the administered dose was excreted in the urine as the glucuronide of cyclopentanol. Small amounts of sulfur-containing metabolites were also detected in the urine representing about 5% of the administered dose. These were reported as an unidentified sulfur-containing metabolite (probably the sulfate ester of hydroxycycloalkylmercapturic acid), an ethereal sulfate and traces of *cis*- and *trans*-2-hydroxycyclopentylmercapturic acid. The unidentified sulfur-containing metabolite and 2-hydroxycyclopentylmercapturic acids were also detected in a similar study with rats (dose not given), but no glucuronide was found. In rats, the addition of glutathione resulting in the formation of 2-hydroxycyclopentylmercapturic acid (Belsito et al., 2012) and other sulfur-containing metabolites appears the main route of excretion. On the other hand, Cronholm (1974) detected in urine and bile of rats the glucuronyl metabolites of about 100% of cyclohexanone 24 h after its administration by gavage.

Metabolism studies of compounds belonging to CG 10 in animals, other than rats and rabbits, are lacking in the scientific literature. Carboxylesterases, responsible for the hydrolysis of esters, are present in the gut especially of ruminants and the liver of several animal species (cattle, pigs, chickens, rabbits and horses), operating the hydrolysis of esters and originating the respective alcohols and acids (Gusson et al., 2006). Carboxylesterase activity also plays a significant role in detoxification processes in fish (Li and Fan, 1997; Di Giulio and Hinton, 2008). Reduction of ketones to alcohols can also be carried out by carbonyl reductases that are widely distributed in animal species, including cattle, pig, rabbit, dog, sheep and birds (Felsted and Bachur, 1980), and more recently evaluated *in vitro* in the liver from cattle, pig, goat and sheep (Szotakova et al., 2004). Oxidative metabolism of xenobiotics is common in all animal species. The CYP450 monooxygenase families are present and have been characterised in a number of food-producing animals, including ruminants, horses, pigs, (Nebbia et al., 2003; Ioannides, 2006; Fink-Gremmels, 2008), fish (Wolf and Wolfe, 2005) and birds (Blevins et al., 2012). All these species also carry out conjugation reactions with sulfate and glucuronic acid (Watkins and Klaassen, 1986; James, 1987; Gusson et al., 2006), producing water-soluble derivatives that are eliminated in urine. Therefore, mammals, fish and birds, can also be assumed to have the ability to metabolise and excrete the flavouring substances from CG 10 and there is no evidence that they or their metabolites would accumulate in tissues and cause a concern for consumer safety. The FEEDAP Panel notes that for feline species the capacity for conjugation is limited (Shrestha et al., 2011; Court, 2013).

3.2.2. Toxicological studies

Subchronic repeated-dose studies with multiple doses tested could be found for 3-hydroxybutan-2-one [07.051], diacetyl [07.052], 3,4-dimethylcyclopentan-1,2-dione [07.075] and 3,5-dimethylcyclopentan-1,2-dione [07.076]. For 3-ethylcyclopentan-1,2-dione [07.057], a chronic study was available. An additional study was identified in which hexan-3,4-dione [07.077] was tested as an admixture with 3-hexanone (15%) at a single dose level. Based on the chemical structure, 3-hydroxybutan-2-one, diacetyl and hexan-3,4-dione are acyclic compounds, the others compounds are cyclopentanediones.

In a 13-week study in rats (males/females, 15 animals/group), 3-hydroxybutan-2-one [07.051] was administered with the diet at doses of 0, 85, 330 and 1,345 mg/kg bw per day. No treatment-related effects on body weight gain, haematological and urinary parameters, serum chemistry, organ weight and histopathology were seen up to 330 mg/kg bw per day. Several effects were observed at the highest dose tested, i.e. a reduction in body weight gain associated with a reduction in food and water consumption, an increase in relative liver weight and a slight anaemia. From this study, a no observed adverse effect level (NOAEL) of 330 mg/kg bw per day could be derived (Gaunt et al., 1972).

A NOAEL of 90 mg/kg bw per day was derived from a 13-week study in rats (15 males/15 females each group), in which diacetyl [07.052] was administered by gavage at nominal doses of 0, 10, 30, 90 and 540 mg/kg bw per day. No adverse effects were seen at the three low doses tested on haematological and urinary parameters, serum chemistry, absolute and relative organ weight and histopathology. Several effects were observed at the highest dose tested (540 mg/kg bw), i.e. a decrease in weight gain associated with an increase in water consumption, anaemia, increased leucocyte count, increased relative weights of the liver, kidneys, adrenals and pituitary glands. At the same dose, stomach lesions seen at necropsy revealed necrosis with infiltration by inflammatory cells (Colley et al., 1969).

A repeated-dose toxicity study (90 days, only one dose tested) in rats was available for hexan-3,4-dione [07.077] containing about 15% 3-hexanone (Posternak et al., 1969). The study considered a number of endpoints (body weight, feed intake; haematology and clinical chemistry; gross pathology and histopathology) and showed no effects at the dose tested, i.e. 17.47 and 17.34 mg/kg bw per day in male and female rats, respectively. The NOAEL for this study is 17.34 mg/kg bw per day, the only dose tested.

The FEEDAP Panel retains a NOAEL of 90 mg/kg bw per day derived from the 90-day study with diacetyl [07.052] and applies it as a group NOAEL for 3-hydroxybutan-2-one [07.051] and its ester [09.186], pentan-2,3-dione [07.060] and hexan-3,4-dione [07.077] on the basis of structure similarity and common metabolism.

A trial was conducted to assess the chronic toxicity of 3-ethylcyclopentan-1,2-dione [07.057] on reproduction and development in rats (male and female Charles River CD-COBS) following administration to three successive generations (King et al., 1979, unpublished). In each generation, rats received diet containing 3-ethylcyclopentan-1,2-dione corresponding to dose levels of 0 (untreated controls), 0 (propylene glycol vehicle), 30, 80, and 200 mg/kg body weight/day. The F0 group (20 animals/sex/treatment) entered the study at weaning and were mated on day 64. Animals from the control groups and the high-dose group were maintained on trial for 12 months. The F1 generation

(50 animals/sex per treatment except control, 100 animals/sex) was exposed to the test substance *in utero*, via milk until weaning and then through the diet for a further 23 months. The final examination of the F1 generation included ophthalmology, clinical chemistry, haematology and a full histopathology. The F1 generation was bred twice (days 99 and 155) and 20 litters/treatment group from the first mating selected to provide the F2 generation which were in turn mated at day 84. The F3 generation were killed after weaning. Survival, food consumption, growth, reproductive performance, haematological and clinical chemistry parameters were not adversely affected. Gross pathological and histopathological examination revealed no significant treatment-related effects. The incidence of benign or malignant tumours in treated animals was not significantly different to that in controls in the F0 and F1 generations. From this study, it is concluded that ethylcyclopentan-1,2-dione [07.057] was not carcinogenic in rats under the study conditions and that a NOAEL of 200 mg/kg body weight (the highest dose tested) can be derived for chronic and developmental effects.

In a 13-week study in male rats (10 animals each group), 3,4-dimethylcyclopentan-1,2-dione [07.075] was administered via the diet at nominal doses of 0, 400, 4,000 and 12,900 mg/kg corresponding to 0, 20, 200 and 645 mg/kg bw per day. The study considered a number of endpoints (mortality, body weight, feed intake; haematology; gross pathology and histopathology). A depression of food intake and a decrease in body weight gain were seen in animals exposed to the highest dose group (> 10% reduction). Since the efficiency of feed conversion was unaffected the authors attributed this to the sensory properties of the diet leading to inappetence. No other changes were observed, which led the authors to retain the highest dose tested as the NOAEL for the study (Wheldon and Krajkeman, 1967, unpublished; RIFM database). In the same report, a NOAEL of 610 mg/kg bw per day was identified for 3,5-dimethylcyclopentan-1,2-dione [07.076]. However, this compound also induced reduced feed intake and growth at the highest dose tested (12,200/24,000 mg/kg diet). Since significant growth reduction is considered adverse the panel opts for the middle dose tested from which a NOAEL of 200 mg/kg body weight can be derived for 3,4-dimethylcyclopentan-1,2-dione and a NOAEL of 500 mg/kg body weight for 3,5-dimethylcyclopentan-1,2-dione.

Secondary references referred to a repeated dose toxicity study (90 days, one dose tested) in rat (15 males/15 females) with 3-methylcyclopentan-1,2-dione [07.056] in which a NOAEL of 500 mg/kg bw per day (corresponding to 1%) was derived (Dow chemical, unpublished, 1953 as described in RIFM report, 1976). However, the study report was not available and the NOAEL could not be confirmed.

The FEEDAP Panel retains the more conservative NOAEL of 200 mg/kg bw per day derived from the combined developmental/carcinogenicity study with 3-ethylcyclopentan-1,2-dione [07.057] and applies it as a group NOAEL for cyclopentanediones.

3.2.3. Safety for the target species

The first approach to the safety assessment for target species takes account of the applied use levels in animal feed relative to the maximum reported exposure of humans on the basis of the metabolic body weight. The data for human exposure in the EU (EFSA, 2008a, 2009, EFSA CEF Panel, 2014b,c) ranges from 0.024 to 2,300 μg/person per day, corresponding to 0.011–106.7 μg/kg$^{0.75}$ per day. Table 3 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 4.

Table 3: Comparison of exposure of humans and target animals to the flavourings under application

EU register name	Use level in feed (mg/kg)	Human exposure (μg/kg bw$^{0.75}$ per day)(1)	Target animal exposure (μg/kg bw$^{0.75}$ per day)		
			Salmon	Piglet	Dairy cow
3-Hydroxybutan-2-one	5	107	118	526	777
Diacetyl	25	102	588	2,632	3,885
3-Methylcyclopentan-1,2-dione	5	26.4	118	526	777
3-Ethylcyclopentan-1,2-dione	5	1.48	118	526	777
Pentan-2,3-dione	5	6.03	118	526	777
3,4-Dimethylcyclopentan-1,2-dione	5	1.39	118	526	777
3,5-Dimethylcyclopentan-1,2-dione	5	1.62	118	526	777
Hexan-3,4-dione	5	0.97	118	526	777
2,6,6-Trimethylcyclohex-2-en-1,4-dione	5	2.32	118	526	777

EU register name	Use level in feed (mg/kg)	Human exposure (μg/kg bw$^{0.75}$ per day)(1)	Target animal exposure (μg/kg bw$^{0.75}$ per day)		
			Salmon	Piglet	Dairy cow
3-Methylnona-2,4-dione	5	0.016	118	526	777
sec-Butan-3-onyl acetate	5	0.0011	118	526	777

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

Table 3 shows that for all compounds the intake by the target animals exceeds that of humans resulting from use in food. As a consequence, safety for the target species at the feed concentration applied cannot be derived from the risk assessment for food use.

As an alternative, the maximum feed concentration considered as safe for the target animal can be derived from the lowest NOAEL available. Toxicological data, from which a NOAEL value could be derived, were available for three acyclic compounds (3-hydroxybutan-2-one [07.051], diacetyl [07.052] and hexan-3,4-dione [07.077]) and four cyclopentadiones (3-methylcyclo-pentan-1,2-dione [07.056], 3-ethylcyclopentan-1,2-dione [07.057], 3,4-dimethylcyclopentan-1,2-dione [07.075] and 3,5-dimethyl cyclopentan-1,2-dione [07.076]) (see Section 3.2.2). For acyclic compounds, a group NOAEL of 90 mg/kg bw per day derived from the 90-day study with diacetyl [07.052] was considered to apply also to 3-hydroxybutan-2-one [07.051] and its ester [09.186], pentan-2,3-dione [07.060] and hexan-3,4-dione [07.077]. For cyclopentanediones, the more conservative NOAEL of 200 mg/kg bw per day derived from the combined developmental/carcinogenicity study with 3-ethylcyclopentan-1,2-dione [07.057] was applied as a group NOAEL.

Applying an uncertainty factor (UF) of 100 to these NOAELs, the maximum safe intake for the target species was derived for the eight compounds following the EFSA Guidance for sensory additives (EFSA FEEDAP Panel, 2012a), and thus the maximum safe feed concentration was calculated. The results are summarised in Table 4. The UF for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation (Court and Greenblatt, 1997).

Table 4: Maximum safe concentration in feed for different target animals for (**A**) acyclic compounds (NOAEL 90 mg/kg bw per day) and (**B**) cyclopentadiones (NOAEL 200 mg/kg bw per day)

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)	
			A	B	A	B
Salmonids	2	40	1.8	4	45	101
Veal calves (milk replacer)	100	2,000	90	200	45	100
Cattle for fattening	400	8,000	360	800	40	88
Dairy cows	650	20,000	585	1,300	26	57
Piglets	20	1,000	18	40	18	40
Pigs for fattening	100	3,000	90	200	30	67
Sows	200	6,000	180	400	30	67
Chickens for fattening	2	120	1.8	4	15	33
Laying hens	2	120	1.8	4	15	33
Turkeys for fattening	12	400	10.8	24	27	60
Dogs	15	250	13.5	30	48	106
Cats(3)	3	60	0.5	1.2	8	18

NOAEL: no observed adverse effect level; bw: body weight.
(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): Complete feed containing 88% DM, milk replacer 94.5% DM.
(3): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.
(**A**): 3-Hydroxybutan-2-one [07.051], diacetyl [07.052], hexan-3,4-dione [07.077], pentan-2,3-dione [07.060] and sec-butan-3-onyl acetate [09.186].
(**B**): 3-Methylcyclopentan-1,2-dione [07.056], 3-ethylcyclopentan-1,2-dione [07.057] 3,4-dimethylcyclopentan-1,2-dione [07.075] and 3,5-dimethylcyclopentan-1,2-dione [07.076].

For the two remaining compounds, 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109] and 3-methylnona-2,4-dione [07.184], adequate subchronic, repeated-dose studies performed with the additive under assessment were not available. Therefore, the threshold of toxicological concern (TTC) approach was followed to derive the maximum safe feed concentration (EFSA FEEDAP Panel, 2012a).

For these two compounds belonging to Cramer Class II compounds, the calculated safe use level for these compounds 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.

3.2.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that for:

- diacetyl [07.052] is safe at the proposed maximum use level of 25 mg/kg complete feed for all target species, except piglets, chickens for fattening, laying hens and cats, for which the proposed normal use level of 5 mg/kg is safe;
- 3-hydroxybutan-2-one [07.051], 3-methylcyclo-pentan-1,2-dione [07.056], 3-ethylcyclopentan-1,2-dione [07.057], pentan-2,3-dione [07.060], 3,4-dimethylcyclopentan-1,2-dione [07.075], 3,5-dimethyl cyclopentan-1,2-dione [07.076], hexan-3,4-dione [07.077] and sec-butan-3-onyl acetate [09.186] are safe at the proposed maximum dose level of 5 mg/kg complete feed for all target species;
- 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109] and 3-methylnona-2,4-dione [07.184] are safe only at concentrations of 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 compounds in CG 10, used as food flavours, has already been assessed by JECFA (WHO, 1999, 2000) and EFSA (EFSA 2008a, 2009; EFSA CEF Panel, 2011, 2014a,b, c). All these compounds are presently authorised as food flavourings without limitations.[5]

Given the use levels of CG 10 compounds to be applied in feed, their hydrophilic properties and the expected extensive metabolism and excretion in target animals (see Section 3.2.1), the FEEDAP Panel considers that the possible residues in food derived from animals fed with these flavourings would not appreciably increase the human intake of these compounds. Consequently, no safety concern would arise for the consumer from the use of these 11 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[14] hazards for skin and eye contact and respiratory exposure are recognised for the majority of the compounds under application. Most are classified as irritating to the respiratory system. In particular, respiratory exposure to diacetyl has been demonstrated to be harmful for exposure at the workplace (review by NIOSH, 2011; Shibamoto, 2014).

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 four substances, namely, 3-hydroxybutan-2-one [07.051], diacetyl [07.052], hexan-3,4-dione [07.077] and 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109], which occur in the environment at levels above the application rate of 25 (for diacetyl) and 5 mg/kg feed for the remaining three compounds (data taken from the Netherlands Organisation for Applied Scientific Research (TNO) database Volatile Compounds in Food *ver.* 14.1; Burdock, 2003).[15]

The other seven compounds, namely 3-methylcyclopentan-1,2-dione [07.056], 3-ethylcyclopentan-1,2-dione [07.057], pentan-2,3-dione [07.060], 3,4-dimethylcyclopentan-1,2-dione [07.075], 3,5-dimethylcyclopentan-1,2-dione [07.076], 3-methylnona-2,4-dione [07.184] and sec-butan-3-onyl acetate [09.186], could not be shown to occur in the environment at levels above the application rate of 5 mg/kg feed for the remaining three compounds. However, the FEEDAP Panel assumes that there is a high

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

probability of complete hydrolysis in the target animal of the ester sec-butan-3-onyl acetate [09.186], resulting in acetic acid and 3-hydroxybutan-2-one [07.051], which are naturally occurring compounds. Similarly, considering the metabolism in the target animals (see Section 3.2.1), the FEEDAP Panel assumes that pentan-2,3-dione [07.060] and 3-methylnona-2,4-dione [07.184] will be completely metabolised in the target animals. Therefore, these compounds are excluded from further assessment.

For the remaining four compounds, namely 3-methylcyclopentan-1,2-dione [07.056], 3-ethylcyclopentan-1,2-dione [07.057], 3,4-dimethylcyclopentan-1,2-dione [07.075] and 3,5-dimethylcyclopentan-1,2-dione [07.076], the predicted environmental concentration for soil (PEC_{soil}) was calculated based on the use rate (Table 5) and compared with the trigger values for compartments set in the phase I of the relevant EFSA guidance (EFSA, 2008b).

Table 5: Predicted environmental concentration (PEC) values of the four flavourings of CG 10 under assessment (calculated for lamb manure)

EU register name	CAS no.	Dose mg/kg	PEC_{soil} (µg/kg)	$PEC_{porewater}$ (µg/L)	$PEC_{surfacewater}$ (µg/L)
3-Methylcyclopentan-1,2-dione	80-71-7	5	107	765	255
3-Ethylcyclopentan-1,2-dione	21835-01-8	5	107	671	224
3,4-Dimethylcyclopentan-1,2-dione	13494-06-9	5	107	788	263
3,5-Dimethylcyclopentan-1,2-dione	13494-07-0	5	107	788	263

EU: European Union; CAS no: Chemical Abstracts Service.

PEC_{soil} values are above the threshold of 10 µg/kg (EFSA, 2008b). The PEC for pore water is dependent on the sorption, which is different for each compound. For these calculations, the substance-dependent constants organic carbon sorption constant (K_{oc}), molecular weight, vapour pressure and solubility are needed. These were estimated from the Simplified Molecular Input Line Entry Specification (SMILES) notation of the chemical structure using EPIWEB 4.1 (Table 6).[16] 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 6: Physicochemical properties predicted by EPIWEB 4.1 for the four flavourings of CG 10 under assessment

EU register name	CAS no.	Predicted by EPIWEB 4.1				
		DT_{50}(1) (days)	Molecular weight (g/mol)	Vapour pressure (Pa)	Solubility (mg/L)	K_{oc}(2) (L/kg)
3-Methylcyclopentan-1,2-dione	80-71-7	7	112.13	0.1	8,501	1.2
3-Ethylcyclopentan-1,2-dione	21835-01-8	7	126.16	0.1	2,878	2.3
3,4-Dimethylcyclopentan-1,2-dione	13494-06-9	10	126.16	25	121,100	1.0
3,5-Dimethylcyclopentan-1,2-dione	13494-07-0	10	126.16	25	121,100	1.0

EU: European Union; CAS no: Chemical Abstracts Service.
(1): DT_{50}: half-life of the additive (EPIWB 4.1.BioWin4.1).
(2): K_{oc}: organic carbon sorption constant (EPIWB 4.1.KocWin2.0).

The half-life (DT_{50}) was calculated using BioWin4.1 (Ultimate Survey Model), which gives a rating number. This rating number *r* was translated into a half-life using the formula by Arnot et al. (2005):

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

This is the general regression used to derive estimates of aerobic environmental biodegradation half-lives from BioWin 4.1 model output.

The calculated predicted concentrations for groundwater ($PEC_{porewater}$) for all four substances are above 0.1 µg/L and for soil (PEC_{soil}) above 10 µg/kg (see Table 5). Therefore, they are subjected to phase II risk assessment.

In the absence of experimental data, the phase II risk assessment was performed using ECOSAR v1.11, which estimates the half-maximal effective concentration (EC_{50}) or lethal concentration (LC_{50}) for ecotoxicologically relevant organisms from the SMILES notation of the substance. The predicted PNEC for

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

aquatic compartment ($PNEC_{aquatic}$) was derived from the lowest toxicity value for freshwater environment by applying a UF of 1,000.

Table 7: Phase II environmental risk assessment of aquatic compartment for CG 10 compounds used as feed additives for terrestrial farm animals (exposure and effect data were modelled using EPIWEB 4.1 and ECOSAR 1.11)

EU Register name Aquatic	LC_{50} (1) Fish (mg/L)	LC_{50} (1) Daphnids (mg/L)	EC_{50} (2) Algae (mg/L)	$PNEC_{aquatic}$ (µg/L)	PEC_{sw} (3) (µg/L)	PEC_{sw}/ $PNEC_{sw}$
3-Methylcyclopentan-1,2-dione	398	331	169	169	255	1.5
3-Ethylcyclopentan-1,2-dione	189	135	76.0	76.1	224	2.9
3,4-Dimethylcyclopentan-1,2-dione	8351	3898	1291	76.1*	263	3.5
3,5-Dimethylcyclopentan-1,2-dione	8351	3898	1291	76.1*	263	3.5

EU: European Union; PNEC: predicted no effect concentration.
(1): LC_{50}: the concentration of a test substance which results in a 50% mortality of the test species.
(2): 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).
(3): PEC_{sw}: predicted environmental concentration in surface water.
*: The LC_{50} for algae of 3-ethylcyclopentan-1,2,dione was taken to derive a PNEC.

For 3,4-dimethylcyclopentan-1,2-dione [07.075] and 3,5-dimethylcyclopentan-1,2-dione [07.076], no proper quantitative structure–activity relationship (QSAR) were available and therefore QSAR for the class 'neutral organic' was used by ECOSAR. This default model is not the most appropriate since these compounds are very similar to the other two cyclopentanediones, 3-methylcyclopentan-1,2-dione [07.056] and 3-ethylcyclopentan-1,2-dione [07.057], for which the vinyl/allylketones QSAR was used. The QSAR for vinyl/alkylketones is considered more relevant for all cyclopentanediones. Therefore, the $PNEC_{aquatic}$ for 3-ethylcyclopentan-1,2,dione [07.057] was taken in preference as a worst-case estimate.

Concerning the fresh water environment, the maximum proposed use level (5 mg/kg) would result in PEC_{sw}/PNEC ratio > 1 for all compounds (Table 7), whereas the proposed normal use level of 1 mg/kg would not cause a risk for this compartment (PEC_{sw}/PNEC ratio in the range 0.301–0.691).

It was not possible to obtain toxicity data for earthworms using ECOSAR for any of the compounds in Table 7. Therefore, the equilibrium partitioning method was applied, which assumes that earthworms do not show higher sensitivity than aquatic organisms. To determine the potential exposure of earthworms, the pore water concentration is set as three times higher than the surface water concentration (EFSA, 2008b). The resulting PEC/PNEC ratio is equal or lower than 1 only when the use level is lower than 0.5 mg/kg.

If used in fish feed at the highest proposed use level of 5 mg/kg complete feed in land-based aquaculture systems, none of the additives under assessment would result in a predicted environmental concentration of the additive (parent compound) in surface water (PEC_{swaq}) above the trigger value of 0.1 µg/L as calculated according to the guidance (EFSA, 2008b). For sea cages, a dietary concentration of 0.047 mg/kg would ensure that the threshold for the predicted environmental concentration of the additive (parent compound) in sediment (PEC_{sed}) of 10 µg/kg is not exceeded when calculated according to the EFSA guidance (EFSA, 2008b).

3.2.6.1. Conclusions on safety for the environment

For 3-hydroxybutan-2-one [07.051], diacetyl [07.052], pentan-2,3-dione [07.060], hexan-3,4-dione [07.077], 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109], 3-methylnona-2,4-dione [07.184] and sec-butan-3-onyl acetate [09.186], the maximum proposed use levels are considered safe for the environment. For cyclopentanediones (3-methylcyclopentan-1,2-dione [07.056], 3-ethylcyclopentan-1.2-dione [07.057], 3,4 dimethylcyclopentan-1,2-dione [07.075] and 3,5-dimethylcyclopentan-1,2-dione [07.076]) usage at levels up to 0.5 mg/kg feed is unlikely to have an adverse effect on the terrestrial or freshwater environments.

3.3. Efficacy

Since all 11 compounds are used in food as flavourings and their function in feed is essentially the same as that in food no further demonstration of efficacy is necessary.

4. Conclusions

The FEEDAP Panel concludes that diacetyl [07.052] is safe at the proposed maximum use level of 25 mg/kg complete feed for all target species, except piglets, chickens for fattening, laying hens and cats, for which the proposed normal use level of 5 mg/kg is safe; 3-hydroxybutan-2-one [07.051], 3-methylcyclo-pentan-1,2-dione [07.056], 3-ethylcyclopentan-1,2-dione [07.057], pentan-2,3-dione [07.060], 3,4-dimethylcyclopentan-1,2-dione [07.075], 3,5-dimethyl cyclopentan-1,2-dione [07.076], hexan-3,4-dione [07.077] and sec-butan-3-onyl acetate [09.186] are safe at the proposed maximum dose level of 5 mg/kg complete feed for all target species; 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109] and 3-methylnona-2,4-dione [07.184], are safe only at concentrations of 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.

No safety concern would arise for the consumer from the use of these compounds up to the highest proposed level in feeds.

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

For 3-hydroxybutan-2-one [07.051], diacetyl [07.052], pentan-2,3-dione [07.060], hexan-3,4-dione [07.077], 2,6,6-trimethylcyclohex-2-en-1,4-dione [07.109], 3-methylnona-2,4-dione [07.184] and sec-butan-3-onyl acetate [09.186], the maximum proposed use levels are considered safe for the environment. For cyclopentanediones (3-methylcyclopentan-1,2-dione [07.056], 3-ethylcyclopentan-1.2-dione [07.057], 3,4 dimethylcyclopentan-1,2-dione [07.075] and 3,5-dimethylcyclopentan-1,2-dione [07.076]) usage at levels up to 0.5 mg/kg feed is unlikely to have an adverse effect on the terrestrial or freshwater environments.

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

Documentation provided to EFSA

1. Chemically defined flavourings from Flavouring Group 10 – Secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group for all animal species and categories. August 2010. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
2. Chemically defined flavourings from Flavouring Group 10 – Secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group for all animal species and categories. Supplementary information. May 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
3. Chemically defined flavourings from Flavouring Group 10 – Secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group for all animal species and categories. Supplementary information. April 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
4. Chemically defined flavourings from Flavouring Group 10 – Secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group for all animal species and categories. Supplementary information. July 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
5. Chemically defined flavourings from Flavouring Group 10 – Secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group for all animal species and categories. Supplementary information. July 2016. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
6. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Chemically Defined Flavourings – Group 10 (CDG 10 – Secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group).
7. Comments from Member States.

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Abbreviations

ADI	acceptable daily intake
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CDG	chemically defined group
DM	dry matter
DT_{50}	degradation half-time
EC_{50}	half-maximal effective concentration
ECOSAR	component program of EPI suite™
EEIG	European Economic Interest Grouping
EPI suite	Estimation Programs Interface (EPI) Suite™
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	the EU Flavour Information System
FL-No	FLAVIS number
GC–MS	gas chromatography–mass spectrometry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
K_{oc}	organic carbon sorption constant
K_{ow}	octanol–water partition coefficient
LC_{50}	lethal concentration 50
Log K_{ow}	logarithm of octanol–water partition coefficient
NOAEL	no observed adverse effect level
PEC	predicted environmental concentration
PEC_{swaq}	predicted environmental concentration of the additive (parent compound) in surface water
PNEC	predicted no effect concentration
QSAR	quantitative structure–activity relationship
SCF	Scientific Committee on Food
SMILES	Simplified Molecular Input Line Entry Specification
TNO	Netherlands Organisation for Applied Scientific Research
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for secondary aliphatic saturated or unsaturated alcohols/ ketones/ketals/esters with a second secondary or tertiary oxygenated functional group

The *Chemically Defined Flavourings - Group 10* (*CDG10 - Secondary aliphatic saturated or unsaturated alcohols/ketones/ketals/esters with a second secondary or tertiary oxygenated functional group*), in this application comprises 11 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 98% (90% for the *3-ethylcyclopentan-1,2-dione*).

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 *CDG10* 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 EURL. The Applicant provided the typical chromatogram for the *CDG10* 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 *CDG10* 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 *CDG10* in the *mixture of flavouring compounds.*

Based on the satisfactory experimental evidence provided, the EURL recommends for official control for the qualitative identification in the *feed additive* of the individual (or mixture of) *flavouring compounds* of interest 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 EURL 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 references Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

(*)Full list provided in EURL evaluation report, available from the EURL website.