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## Safety and efficacy of oct-1-en-3-ol, pent-1-en-3-ol, oct-1-en-3-one, oct-1-en-3-yl acetate, isopulegol and 5-methylhept-2-en-4-one, belonging to chemical group 5 and of isopulegone and $\alpha$ -damascone belonging to chemical group 8 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 oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081], oct-1-en-3-yl acetate [09.281], isopulegol [02.067] and 5-methylhept-2-en-4-one [07.139], belonging to chemical group 5, and of isopulegone [07.067] and  $\alpha$ -damascone [07.134] belonging to chemical group 8, when used as feed flavourings for all animal species. They are currently authorised for use as flavours in food. The FEEDAP Panel was unable to assess the safety of isopulegone because the purity of the compound could not be established and the safety of  $\alpha$ -damascone [07.134] because of the inconclusive assessment of its genotoxicity. The FEEDAP Panel concluded that the use of isopulegol [02.067] is safe at the maximum proposed dose of 5 mg/kg complete feed for all animal species, except cats for which the use level of 1 mg/kg is considered safe. 5-Methylhept-2-en-4-one [07.139] is safe at the proposed normal use levels of 1 mg/kg complete feed for all animal species. For oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081] and oct-1-en-3-yl acetate [09.281], the calculated safe use level is 1 mg/kg complete feed for all animal species, except cats for which the calculated safe level is 0.6 mg/kg. No safety concern would arise for the consumer from the use of these compounds up to the highest safe levels in feed. In the absence of studies to assess the safety for the user, the FEEDAP Panel cannot conclude on the safety for the users when handling the additives. Use of the compounds in animal feed at the maximum safe level is considered safe for the environment. Since the compounds are used in food as flavourings and their function in feed is essentially the same, no demonstration of efficacy is necessary.

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**Keywords:** sensory additives, feed flavourings, secondary alcohols, ketones and esters, chemical group 5, chemical group 8, safety

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## 1. Introduction

### 1.1. Background and Terms of Reference

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

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)<sup>2</sup> for authorisation of 24 substances belonging to chemical group (CG) 5, when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). CG 5 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000<sup>3</sup> 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'. During the course of the assessment, this application was split and the present opinion covers only six out of the 24 substances under application (see Section 1.2).

In addition, the European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)<sup>2</sup> for authorisation/re-evaluation of 32 substances belonging to CG 8, when used as feed additives for all animal species (category: sensory additives; functional group: flavouring compounds). CG 8 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000 as 'secondary alicyclic saturated and unsaturated alcohols/ketones/ketals/esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols. Esters may contain aliphatic acyclic or alicyclic acid component'. During the course of the assessment, this application was split and the present opinion covers two out of the 32 substances under application (see Section 1.2).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the applications to the European Food Safety Authority (EFSA) as applications 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.<sup>4</sup> EFSA received directly from the applicant the technical dossiers in support of these applications. The particulars and documents in support of the applications on CG 5 and CG 8 were considered valid by EFSA as of 20 September 2010 and 21 November 2010, respectively.

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 additives comply 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 oct-1-en-3-ol (EU Flavour Information System (FLAVIS) number) [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081], oct-1-en-3-yl acetate [09.281], isopulegol [02.067] and 5-methylhept-2-en-4-one [07.139], belonging to chemical group 5, and of isopulegone [07.067] and  $\alpha$ -damascone [07.134], belonging to chemical group 8, when used under the proposed conditions of use (see Section 3.1.3).

### 1.2. Additional information

The initial application on CG 5 concerned 24 compounds assigned to this CG, intended to be used as feed flavourings for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has already delivered an opinion on 17 of the 24 compounds of CG 5

<sup>1</sup> 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.

<sup>2</sup> On 13/3/2013, 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.

<sup>3</sup> 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.

<sup>4</sup> 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.

(EFSA FEEDAP Panel, 2015). During the assessment, the applicant withdrew the application for 6,10-dimethyl-5,9-undecadien-2-one [07.216].

The remaining six compounds were excluded from the previous assessment because the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) had requested additional toxicity data to complete their assessment (EFSA, 2009; EFSA CEF Panel, 2012a,b). The EFSA CEF Panel has delivered three opinions, dealing with five of the compounds. For oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081] and oct-1-en-3-yl acetate [09.281], the EFSA CEF Panel concluded that the genotoxicity concerns could be ruled out (EFSA CEF Panel, 2016a) and that there is 'no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach' (EFSA CEF Panel, 2016b). For (1*R*,2*S*,5*R*)-isopulegol [02.067], the applicant submitted a 90-day oral toxicity study which allowed the CEF Panel to agree with JECFA conclusions on isopulegol and two related substances, (2*R*,5*S*)-isopulegone and (1*R*,2*S*,5*R*)-isopulegyl acetate (EFSA CEF Panel, 2017). For 5-methylhept-2-en-4-one [07.139], the EFSA Panel on Food Additives and Flavourings (EFSA FAF Panel) concluded that the genotoxicity concerns could be ruled out (EFSA FAF Panel, 2019a).

Similarly, the initial application on CG 8 concerned 32 compounds assigned to this CG, intended to be used as feed flavourings for all animal species. The FEEDAP Panel has already delivered an opinion on 29 of the 32 compounds of CG 8 (EFSA FEEDAP Panel, 2016).

The EFSA CEF Panel raised concerns for two compounds, requesting additional toxicity data for isopulegone [07.067] (EFSA, 2009) and genotoxicity data for  $\alpha$ -damascone [07.134] (EFSA CEF Panel, 2014, 2015). The 90-day study submitted for (1*R*,2*S*,5*R*)-isopulegol was considered to cover also the structurally related (2*R*,5*S*)-isopulegone [07.067] (EFSA CEF Panel, 2017). For  $\alpha$ -damascone [07.134], the EFSA FAF Panel concluded that the concern for genotoxicity cannot be ruled out (EFSA FAF Panel, 2019b).

d,l-Menthol [02.218] was excluded from the previous assessment as during the assessment the applicant expressed the intention to withdraw the application for this compound.<sup>5</sup> On 7 March 2017, the applicant clarified that d,l-menthol [02.218] and menthol [02.015] are the same additive. The same FLAVIS number [02.015] can be adequately used to identify the racemate and its isomeric forms. Therefore, d,l-menthol [02.218] does not need to be further assessed in the present opinion.

Consequently, this opinion deals with the eight compounds, namely oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081], oct-1-en-3-yl acetate [09.281], isopulegol [02.067] and 5-methylhept-2-en-4-one [07.139], belonging to chemical group 5, and isopulegone [07.067] and  $\alpha$ -damascone [07.134] belonging to chemical group 8, all excluded from the previous opinions.

The eight compounds are currently listed in the European Union database of flavouring substances<sup>6</sup> 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.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of technical dossiers<sup>7</sup> in support of the authorisation request for the use of the compounds under assessment as feed additives.

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:

- Chemical group 5 – saturated and unsaturated aliphatic secondary alcohol/ketones/esters with esters containing secondary alcohols. No aromatic or heteroaromatic moiety as a component of

<sup>5</sup> The applicant informed EFSA (12 September 2011) on the intention to withdraw the application for d,l-menthol [FLAVIS Number 02.218].

<sup>6</sup> 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.

<sup>7</sup> FEED dossier reference: FAD-2010-0412 and FAD-2010-0414.

an ester or ketal – in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>8</sup>

- Chemical group 8 – secondary alicyclic saturated and unsaturated alcohols/ketones/ketals/esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols. Esters may contain aliphatic acyclic or alicyclic acid component – in animal feed. The Executive Summary of the EURL report can be found in Annex B.<sup>9</sup>

## 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<sup>10</sup> and the relevant guidance documents<sup>11</sup>: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA 2008), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

## 3. Assessment

The eight additives under assessment are six compounds belonging to chemical group 5 – ‘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’ and two compounds belonging to chemical group 8 – ‘secondary alicyclic saturated and unsaturated alcohols/ketones/ketals/esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols. Esters may contain aliphatic acyclic or alicyclic acid component’, intended for use as sensory additives (functional group: flavouring compounds) in feed for all animal species.

### 3.1. Characterisation

#### 3.1.1. Characterisation of the flavouring substances

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

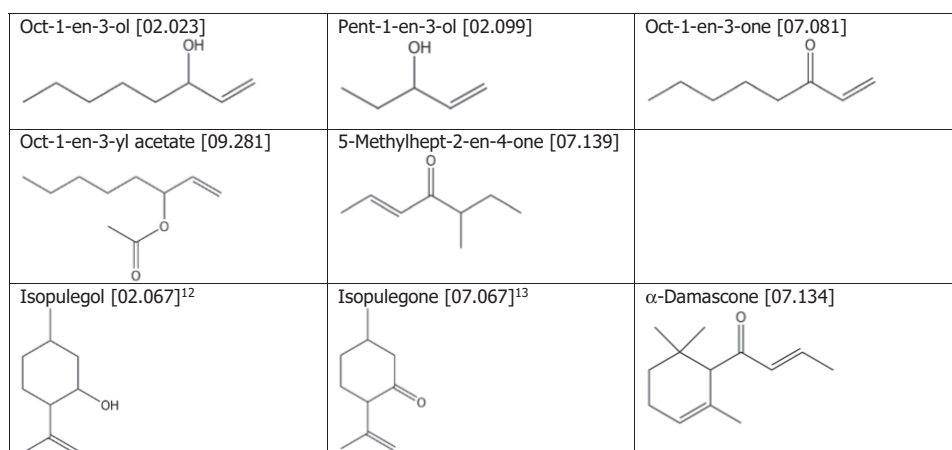
<sup>8</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0074.pdf>

<sup>9</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0125.pdf>

<sup>10</sup> 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.

<sup>11</sup> The Guidance documents in force in 2011–2012 were exceptionally applied to the present assessment to ensure consistency in the assessment of feed flavourings (re-evaluation of authorised feed additives).





**Figure 1:** Molecular structures, EU register names and [FLAVIS numbers] of the eight flavouring compounds under assessment

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

EU register name	CAS no	Flavis no	Molecular formula	Molecular weight	Physical state	Log K <sub>ow</sub>
Oct-1-en-3-ol	3391-86-4	02.023	C <sub>8</sub> H <sub>16</sub> O	128.21	Liquid	2.64
Pent-1-en-3-ol	616-25-1	02.099	C <sub>5</sub> H <sub>10</sub> O	86.13	Liquid	1.12
Oct-1-en-3-one	4312-99-6	07.081	C <sub>8</sub> H <sub>14</sub> O	126.2	Liquid	2.17
Oct-1-en-3-yl acetate	2442-10-6	09.281	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	170.25	Liquid	3.47
5-Methylhept-2-en-4-one	81925-81-7	07.139	C <sub>8</sub> H <sub>14</sub> O	126.19	Liquid	1.97
Isopulegol	89-79-2	02.067	C <sub>10</sub> H <sub>18</sub> O	154.25	Liquid	3.37
Isopulegone	29606-79-9	07.067	C <sub>10</sub> H <sub>16</sub> O	152.24	Liquid	2.47
$\alpha$ -Damascone	43052-87-5	07.134	C <sub>13</sub> H <sub>20</sub> O	192.3	Liquid	4.40

All of the compounds under consideration are produced by chemical synthesis and typical routes of synthesis are described for each compound.<sup>14</sup>

Data were provided on the batch to batch variation in five batches of each additive except for isopulegone [07.067].<sup>15</sup> Since no data were provided on the purity of isopulegone, this compound was excluded from further assessment. For the remaining seven compounds, 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 % <sup>(1)</sup>	Assay %	
			Average	Range
Oct-1-en-3-ol	02.023	96	99.6	99.5–99.8
Pent-1-en-3-ol	02.099	98	99.3	98.8–99.7
Oct-1-en-3-one	07.081	96	99.2 <sup>(2)</sup>	99.0–99.5
Oct-1-en-3-yl acetate	09.281	95	99.4	99.0–99.8
5-Methylhept-2-en-4-one	07.139	98	99.2	99.2–100
Isopulegol	02.067	95 <sup>(3)</sup>	97.5	95.6–100
Isopulegone	07.067	95	n.a. <sup>(4)</sup>	n.a.

<sup>12</sup> Isopulegol [02.067]: Referred in the register as to (1*R*,2*S*,5*R*)-Isopulegol.

<sup>13</sup> Isopulegone [07.067]: Referred in the register as to (2*R*,5*S*)-Isopulegone.

<sup>14</sup> Technical dossiers FAD-2010-0412 and FAD-2010-0414/Section II.

<sup>15</sup> Technical dossier FAD-2010-0412/Section II/Annex 2.1 and Supplementary information June 2011 and Technical dossier FAD-2010-0414/Section II/Annex 2.1 and Supplementary information September 2011.

EU register name	Flavis no	JECFA specification minimum % <sup>(1)</sup>	Assay %	
			Average	Range
$\alpha$ -Damascone	07.134	98 <sup>(5)</sup>	98.7	98.2–99.9

(1): FAO (2006).

(2): Four batches; the fifth batch is diluted to 50 wt. % in 1-octen-3-ol.

(3): Total sum of isomers; < 1% citronellal.

(4): No data available for this substance.

(5): Sum of cis and trans isomers, 92–96% trans, 4–8% cis isomer.

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 isopulegol [02.067] 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.<sup>16</sup> 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 seven compounds, namely oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081], oct-1-en-3-yl acetate [09.281], isopulegol [02.067], 5-methylhept-2-en-4-one [07.139] and  $\alpha$ -damascone [07.134], in feed for all animal species without withdrawal. For all additives, the applicant proposes a normal use level of 1 mg/kg feed and a high use level of 5 mg/kg feed.

## 3.2. Safety

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

The compounds under assessment have been recently evaluated by EFSA as food flavourings (EFSA CEF Panel, 2016a,b, 2017, EFSA FAF Panel et al., 2019a,b), with a positive outcome (no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach) for all except  $\alpha$ -damascone.

Since the FAF Panel concluded that the concern for genotoxicity cannot be ruled out for  $\alpha$ -damascone [07.134] (EFSA FAF Panel, 2019b), the FEEDAP Panel cannot conclude that the additive is safe when used as a feed flavouring.

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

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 (linear and alicyclic) and esters 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; (iii) reduction of ketones to the corresponding secondary alcohol with subsequent excretion as conjugate of glucuronic acid; (iv) oxidation of double bonds; (v) conjugation with glutathione, (vi) hydrolysis of esters via carboxylesterases 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.

Oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099] and oct-1-en-3-one [07.081] are expected to be oxidised at the terminal unsaturation. The epoxides formed will be hydrolysed and the resulting primary alcohol will be oxidised to form a carboxylic acid, which will further decarboxylate. The

<sup>16</sup> Technical dossier FAD-2010-0412/Supplementary information June 2011 and Technical dossier FAD-2010-0414/Supplementary information September 2011.



remaining  $\alpha$ -keto-alcohol will be further oxidised and then coupled with CoA, followed by the standard degradation of fatty acids.

The major metabolite of isopulegol [02.067] is its glucuronide-conjugate. Oxidation of isopulegol leads to isopulegone [07.067]. Isopulegone is further hydroxylated to 9-hydroxyisopulegone, an intermediate metabolite in the formation of menthofuran (Adams and Taylor, 2009). However, this metabolite is formed only in trace amounts and this pathway is not relevant at the intended levels for feed use. According to experiments performed in humans (Engel, 2003) and rats (Chen et al., 2003), no interconversion between pulegone and isopulegone takes place (EFSA CEF Panel, 2017).

Studies of metabolism of compounds belonging to CGs 5 and 8 in animals other than rodents are lacking in the scientific literature.<sup>17</sup> However, the enzymes involved in the biotransformation pathways of these compounds are present in all target species (reviewed in EFSA FEEDAP Panel, 2015, 2016). Therefore, food-producing animals, including fish and birds, as well as non-food producing animals can also be assumed to have the ability to metabolise and excrete the flavourings under assessment.

### 3.2.2. Toxicological studies

Subchronic studies or other repeated-dose studies with multiple doses tested were submitted only for isopulegol [02.067].

An Organisation for Economic Co-operation and Development (OECD) 408 compliant 90-day study with isopulegol (20%) microencapsulated in 80% acacia gum (Koetzner, 2013) was assessed by the EFSA CEF Panel (EFSA CEF Panel, 2016a). Four groups of rats (10/sex per dietary intake level) were fed a diet providing daily intakes of microencapsulated isopulegol-containing acacia gum 0, 190, 1,750 and 3,500 mg/kg body weight (bw) for males and 0, 190, 1,760 and 3,530 mg/kg bw for females, respectively. There were no effects attributable to isopulegol administration on mortality, clinical or ophthalmological changes, clinical chemistry or urinalysis parameters. Based on a dose-related decrease (36%) in eosinophils observed in males, which reached statistical significance in the highest dose group and on an increase in relative kidney weights in males administered the two highest dietary doses, the EFSA CEF Panel concluded that only the lowest dose in male rats provides a no observed adverse effect level (NOAEL) of 190 mg test item/kg bw per day, corresponding to 38 mg isopulegol/kg bw per day. The FEEDAP Panel supports the conclusions of the CEF Panel.

A repeated-dose toxicity study (90 days, only one dose tested) in rats was available with oct-1-en-3-one [07.081] (Cox et al., 1974, unpublished; LINK). The study considered a number of endpoints (survival, behaviour, body weight, feed intake; haematology, clinical chemistry and urine analysis<sup>18</sup>; gross pathology and histopathology) was properly reported and showed no effects at the dose tested. For these reasons, the FEEDAP Panel concluded that from this study, an NOAEL of 6.7 mg/kg bw per day could be derived for oct-1-en-3-one [07.081]. However, it should be noted that only one dose was tested leaving a great deal of uncertainty regarding the precision of this value.

Secondary references referred to a repeated-dose toxicity study (90 days, one dose tested) in rat for oct-1-en-3-ol [02.023] (Posternak, 1964, unpublished). The study report is not available and the FEEDAP Panel is unable to confirm the NOAEL derived of 12 mg/kg bw per day.

Because of similarity in structure and metabolism, the FEEDAP Panel considers that the NOAEL identified for oct-1-en-3-one [07.081] can be extrapolated to oct-1-en-3-ol [02.023], oct-1-en-3-yl acetate [09.281] and pent-1-en-3-ol [02.099].

### 3.2.3. Safety for the target species

The maximum feed concentration which can be considered safe for the target animals can be derived from the lowest NOAEL identified, if suitable data are available (EFSA FEEDAP Panel, 2012a).

Toxicological data derived from a subchronic study were available for isopulegol [02.067] and oct-1-en-3-one [07.081] (see Section 3.2.2). The NOAEL of 6.7 mg/kg bw per day for oct-1-en-3-one [07.081] is considered to apply also to oct-1-en-3-ol [02.023] and oct-1-en-3-yl acetate [09.281] because they share common metabolic pathways and are interconvertible by hydrolysis and oxidation/reduction reactions, and to pent-1-en-3-ol [02.099]. 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

<sup>17</sup> Technical dossiers FAD-2010-0412 and FAD-2010-0414/Section III.

<sup>18</sup> Haematology: total and differential leucocyte counts, erythrocytes, haemoglobin, haematocrit; clinical chemistry: serum urea nitrogen, blood glucose, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase and serum alkaline phosphatase; urine analysis: pH, specific gravity, qualitative test for albumin, glucose, occult blood and microscopic examination of the centrifuged urinary sediment.

EFSA Guidance for sensory additives (EFSA FEEDAP Panel, 2012a), and thus, the maximum safe feed concentration was calculated (Table 3).

Because glucuronidation 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, because cats have an unusually low capacity for glucuronidation (Court and Greenblatt, 1997).

**Table 3:** Maximum safe concentration in feed for different target animals for (A) isopulegol [02.067] and (B) oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081] and oct-1-en-3-yl acetate [09.281]

Target animal	Default values		Maximum safe intake/feed concentration			
	Body weight (kg)	Feed intake (g/day) <sup>(1)</sup>	Intake (mg/day)		Concentration (mg/kg feed) <sup>(2)</sup>	
			A	B	A	B
Salmonids	2	40	0.76	0.13	19	3.4
Veal calves (milk replacer)	100	2,000	38	6.7	19	3.4
Cattle for fattening	400	8,000	152	26.8	17	2.9
Dairy cows	650	20,000	247	43.6	11	1.9
Piglets	20	1,000	7.6	1.34	8	1.3
Pigs for fattening	100	3,000	38	6.7	13	2.2
Sows	200	6,000	76	13.4	13	2.2
Chickens for fattening	2	120	0.76	0.13	6	1.1
Laying hens	2	120	0.76	0.13	6	1.1
Turkeys for fattening	12	400	4.6	0.80	11	2.0
Dogs	15	250	5.7	1.01	20	3.5
Cats	3	60	0.23	0.04	3.4 <sup>(3)</sup>	0.6 <sup>(3)</sup>

(1): Complete feed with 88% DM, except milk replacer for veal calves (94.5% DM), and for cattle for fattening, dairy cows, dogs and cats for which the values are DM intake.

(2): 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.

Since an individual reliable NOAEL could not be found for 5-methylhept-2-en-4-one [07.139], the threshold of toxicological concern (TTC) approach was followed to derive the maximum safe feed concentration (EFSA FEEDAP Panel, 2012a). For this Cramer class I compound, the calculated safe use level is 1.5 mg/kg complete feed for cattle, salmonids and non-food producing animals and 1.0 mg/kg complete feed for pigs and poultry.

### Conclusions on safety for the target species

The FEEDAP Panel concludes that:

- isopulegol [02.067] is safe at the maximum proposed dose of 5 mg/kg complete feed for all animal species, except cats for which the use level of 1 mg/kg is considered safe;
- 5-methylhept-2-en-4-one [07.139] is safe at the proposed normal use levels of 1 mg/kg complete feed for all animal species;
- oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081] and oct-1-en-3-yl acetate [09.281] are safe at the use level of 1 mg/kg complete feed for all animal species, except cats for which the calculated maximum safe level is 0.6 mg/kg.

Since a concern for genotoxicity cannot be ruled out for  $\alpha$ -damascone [07.134], the FEEDAP Panel is unable to assess the safety of this compound when used as a feed flavouring.

### 3.2.4. Safety for the consumer

The safety for the consumer of the six compounds used as food flavours has been already assessed by JECFA (WHO, 2000, 2001) and EFSA (EFSA CEF Panel, 2016a, 2016b, 2017; EFSA FAF Panel, 2019a,b). All compounds are currently authorised in the EU as food flavourings without limitations.<sup>6</sup>

Although deposition and residue studies of the compounds in farm animals are not available, the FEEDAP Panel considers that the use of these flavourings in animal feed would not appreciably increase the human exposure to these compounds. This is based on the low use levels to be applied in

feed and the expected extensive metabolism and excretion in target animals (see Section 3.2.1). Consequently, no safety concern would arise for the consumer from the use of these six compounds up to the highest levels considered safe for target animals (1 or 5 mg/kg complete feed).

### 3.2.5. Safety for the user

The applicant produced a safety data sheet<sup>19</sup> for each compound where hazards for users have been identified. No specific data to assess the safety for the user were submitted. Therefore, the FEEDAP Panel cannot conclude on the safety for users when handling the additive.

### 3.2.6. Safety for the environment

The compounds under assessment are naturally occurring in the environment<sup>20</sup> or expected to be fully metabolised to carbon dioxide and water either in the animal (see Section 3.2.1) or subsequently in the environment. Consequently, their use in animal nutrition at the concentrations in feed considered safe for the target species is also considered safe for the environment.

## 3.3. Efficacy

Since all the 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 was unable to assess the safety of isopulegone because the purity of the compound could not be established. Since a genotoxicity concern could not be ruled out for  $\alpha$ -damascone [07.134], the FEEDAP Panel was also unable to conclude on the safety of this compound when used as a feed flavouring.

The use of isopulegol [02.067] is safe at the maximum proposed dose of 5 mg/kg complete feed for all animal species, except cats for which the use level of 1 mg/kg is considered safe. 5-Methylhept-2-en-4-one [07.139] is safe at the proposed normal use levels of 1 mg/kg complete feed for all animal species. For oct-1-en-3-ol [02.023], pent-1-en-3-ol [02.099], oct-1-en-3-one [07.081] and oct-1-en-3-yl acetate [09.281], the calculated safe use level is 1 mg/kg complete feed for all animal species, except cats, for which the calculated safe level is 0.6 mg/kg.

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

In the absence of studies to assess the safety for the user, the FEEDAP Panel cannot conclude on the safety for users when handling the additives.

The use of the six compounds in animal feed at the respective maximum safe use level 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.

## Documentation provided to EFSA/Chronology

Date	Event
29/07/2010	Dossier received by EFSA. 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). Submitted by FEFANA Asbl/Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
09/08/2010	Reception mandate from the European Commission
20/09/2010	Application validated by EFSA – Start of the scientific assessment

<sup>19</sup> Technical dossier FAD-2010-0412/Section II/Annex II.3. Hazards for skin and eye contact are recognised for oct-1-en-3-ol [02.023], isopulegol [02.067], oct-1-en-3-one [07.081] and 5-methylhept-2-en-4-one [07.139]. Hazard for respiratory exposure is recognised for oct-1-en-3-ol [02.023] and isopulegol [02.067]. 5-Methylhept-2-en-4-one [07.139] and oct-1-en-3-yl acetate [09.281] are also reported to be skin sensitizers.

<sup>20</sup> Technical dossier FAD-2010-0412/Supplementary information June 2011. Data taken from the TNO database Volatile Compounds in Food ver. 14.1; Burdock, 2009.

Date	Event
01/10/2010	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for target species, safety for the consumer, safety for the user and efficacy</i>
21/12/2010	Comments received from Member States
28/01/2011	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
19/07/2011	Request of supplementary/complementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 (addendum) – Scientific assessment remains suspended <i>Issues: safety for target species</i>
22/06/2012	Request of supplementary/complementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 (addendum) – Scientific assessment remains suspended <i>Issues: Safety for the consumer</i>
10/03/2016	Partial withdrawal from EC: use in water (Art. (4))
21/10/2015	The applicant was informed that the application was split into two separate questions with two different EFSA-Q-numbers. The initial EFSA-Q-2010-01040 was assigned to the 18 compounds for which EFSA has completed the evaluation as food flavours, whereas the new EFSA-Q-2015-00599 was assigned to the 6 compounds, for which the assessment was pending
26/08/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
10/01/2020	Opinion adopted by written procedure by the FEEDAP Panel. End of the Scientific assessment
14/09/2010	Dossier received by EFSA. Chemically defined flavourings from Flavouring Group 08 - secondary alicyclic saturated and unsaturated alcohols/ketones/ketals/esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols. Esters may contain aliphatic acyclic or alicyclic acid component (CDG 08). Submitted by FEFANA Asbl/Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
11/10/2010	Reception mandate from the European Commission
02/12/2010	Application validated by EFSA – Start of the scientific assessment
04/01/2011	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for target species, safety for the consumer, safety for the user and efficacy</i>
28/02/2011	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
25/02/2011	Comments received from Member States
29/11/2011	Request of supplementary/complementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 (addendum) – Scientific assessment remains suspended <i>Issues: safety for target species</i>
10/03/2016	Partial withdrawal from EC: use in water (Art. (4))
02/03/2016	The applicant was informed that the application was split into two separate questions with two different EFSA-Q-numbers. The initial EFSA-Q-2010-01181 was assigned to the 29 compounds for which EFSA has completed the evaluation as food flavours, whereas the new EFSA-Q-2016-00599 was assigned to the 3 compounds, for which the assessment was pending
26/08/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
10/01/2020	Opinion adopted by written procedure by the FEEDAP Panel. End of the Scientific assessment

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## Abbreviations

AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
bw	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
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organisation
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FFAC	Feed Flavourings authorisation Consortium of FEFANA (EU Association of Speciality Feed Ingredients and their Mixtures)
FGE	food group evaluation
FLAVIS	The EU Flavour Information System
GC-MS	gas chromatography-mass spectrometry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
Log Kow	logarithm of octanol-water partition coefficient
MSDI	maximised survey-derived daily intake
bw <sup>0.75</sup>	metabolic body weight
NOAEL	no observed adverse effect level
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organisation



## **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 24 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 20 chemically defined flavourings belonging to 20 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.

## **Annex B – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Secondary alicyclic saturated and unsaturated alcohols/ ketones/ketals/esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols**

The *Chemically Defined Flavourings - Group 08 (Secondary alicyclic saturated and unsaturated alcohols/ketones/ketals/esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols)*, in this application comprises 32 substances, for which authorisation as feed additives is sought under the category 'sensory additives', functional group 2(b) 'flavouring compounds', according to the classification system of Annex I of Regulation (EC) No 1831/2003.

In the current application submitted according to Article 4(1) and Article 10(2) of Regulation (EC) No 1831/2003, the authorisation for all species and categories is requested. The flavouring compounds of interest have a purity ranging from 90% to 99% and 85% for methyl 3-oxo-2-pentyl-1-cyclopentylacetate.

*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 *CDG08* 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 *CDG08* 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 20 chemically defined flavourings belonging to 20 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 *CDG08* 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 *CDG08* in the *mixture of flavouring compounds*.

Based on the satisfactory experimental evidence provided, the EURL recommends for official control for the qualitative identification in the *feed additive* of the individual (or mixture of) *flavouring compounds* of interest (\*) the GC-MS-RTL (Agilent specific) method submitted by the Applicant. However, the method is not able to discriminate between [menthol & D-menthol] or [ $\beta$ -Damascone & tr-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)but-2-en-1-one] or the two isomers [d-Carvone & l-Carvone].

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

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