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# Safety and efficacy of aromatic ketones, secondary alcohols and related esters belonging to chemical group 21 when used as flavourings for all animal species

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

## Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of 10 compounds belonging to chemical group 21 (aromatic ketones, secondary alcohols and related esters). They are currently authorised as flavours in food. This opinion concerns eight compounds from this group. The FEEDAP Panel established the following conclusions: 4-(4-hydroxyphenyl)butan-2-one [07.055] is safe at 25 mg/kg complete feed for all target species except chickens for fattening, laying hens and cats, for which 5 mg/kg is considered safe; acetophenone [07.004] at 25 mg/kg for salmonids, veal calves and dogs, and at 5 mg/kg for the remaining target species (4.4 mg/kg for cats); vanillyl acetone [07.005] and 4-(4-methoxyphenyl)-butan-2-one [07.029] at 5 mg/kg for all target species; 1-phenylethan-1-ol [02.064], 4-methylacetophenone [07.022], 4-methoxyacetophenone [07.038] and 1-phenethyl acetate [09.178] at 5 mg/kg for all species, except cats for which 1 mg/kg is considered safe. 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. The proposed maximum use levels in feed are unlikely to have detrimental effects on the terrestrial and fresh water environments, with the exceptions of 4-(4-hydroxyphenyl)butan-2-one [07.055] and 1-phenethyl acetate [09.178] for which the normal use levels are considered safe. For 1-phenylethan-1-ol [02.064], it was not possible to reach a conclusion on the safety for the terrestrial compartment. 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, feed flavourings, aromatic ketones, secondary alcohols, related esters, chemical group 21, safety

**Requestor:** European Commission

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**Amendment:** An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the older version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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# 1. Introduction

## 1.1. Background and Terms of Reference

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

The European Commission (EC) received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation of 10 substances (1-phenylethan-1-ol, acetophenone, vanillyl acetone, 4-methylacetophenone, 4-phenylbut-3-en-2-one, 4-(4-methoxyphenyl)-butan-2-one, benzophenone, 4-methoxyacetophenone, 4-(p-hydroxyphenyl)-butan-2-one (hereafter referred as 4-(4-hydroxyphenyl)-butan-2-one) and 1-phenethyl acetate) belonging to chemical group (CG) 21, when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). CG 21 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[3] as 'aromatic ketones, secondary alcohols and related esters'. During the course of the assessment, this application was split and the present opinion covers eight out of the 10 substances under application (see Section 1.2).

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 20 September 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 1-phenylethan-1-ol (EU Flavour Information System (FLAVIS) number) [02.064], acetophenone [07.004], vanillyl acetone [07.005], 4-methylacetophenone [07.022], 4-(4-methoxyphenyl)-butan-2-one [07.029], 4-methoxyacetophenone [07.038], 4-(4-hydroxyphenyl) butan-2-one [07.055] and 1-phenethyl acetate [09.178], when used under the proposed conditions of use (see Section 3.1.3).

## 1.2. Additional information

All 10 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 2001, 2002) and were considered safe for use in food. An acceptable daily intake (ADI) value was established for 1-phenylethan-1-ol [02.064].

The EFSA Panel on Food Additive, Flavourings, Processing Aids and Materials in Contact with Food (CEF) agreed with JECFA conclusions for nine of the ten compounds (EFSA, 2008a,b), but raised a concerns for genotoxicity for 4-phenylbut-3-en-2-one [07.024] and requested additional genotoxicity data (EFSA, 2008c; EFSA CEF Panel, 2014). The International Agency for Research on Cancer (IARC) published a monograph on benzophenone [07.032] indicating that there was sufficient evidence of carcinogenicity in animals and expressing the possibility of carcinogenicity in humans (IARC, 2013). This was ascribed either to oxidative damage or to endocrine-disrupting effects. In response, the EC

[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 13/3/2013, 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.

[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.

has requested EFSA to review the data concerning the safety of benzophenone. Consequently, the FEEDAP Panel will not proceed with an assessment of these two compounds until the outstanding issues have been addressed.

The current assessment concerns eight compounds, all of which are all currently listed in the European Union database of flavouring substances[5] and in the European Union Register of Feed Additives, respectively, and thus authorised for use in food and feed in the European Union. They have not been previously assessed by EFSA as feed additives.

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

# 2. Data and methodologies

## 2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[7] in support of the authorisation request for the use of aromatic ketones, secondary alcohols and related esters 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 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 'aromatic ketones, secondary alcohols and related esters 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, 2008d), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

# 3. Assessment

## 3.1. Characterisation

### 3.1.1. Characterisation of the flavouring additives

The molecular structures of the eight flavouring additives under application are shown in Figure 1 and their physico-chemical 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-2010-0075.

[8] The full report is available on the EURL website http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0075.pdf

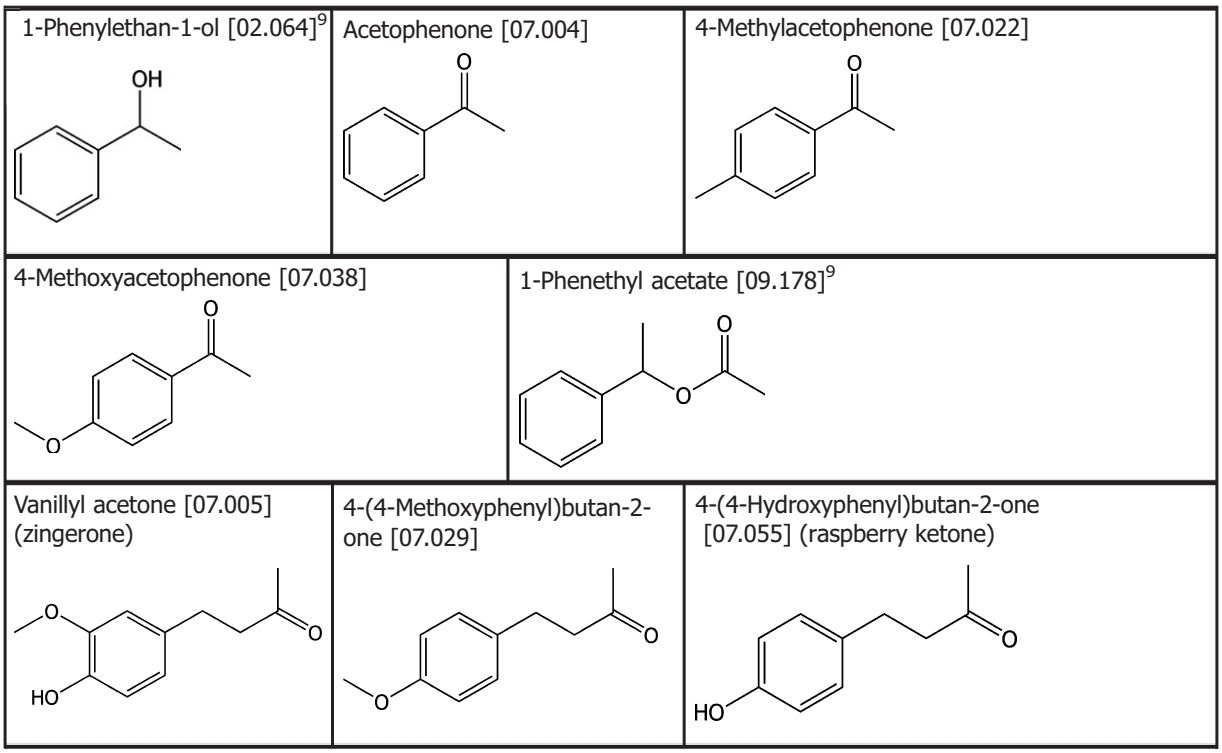


**Figure 1:** Molecular structures, [FLAVIS numbers] and (trivial names) of the eight flavouring compounds under assessment

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

| EU register name | CAS no. | FLAVIS no. | Molecular formula | Molecular weight | Physical state | Log $K_{ow}$[(a)] |
|---|---|---|---|---|---|---|
| 1-Phenylethan-1-ol | 98-85-1 | 02.064 | $C_8H_{10}O$ | 122.17 | Liquid | 1.49 |
| Acetophenone | 98-86-2 | 07.004 | $C_8H_8O$ | 120.15 | Liquid | 1.58 |
| Vanillyl acetone | 122-48-5 | 07.005 | $C_{11}H_{14}O_3$ | 194.23 | Solid | 1.31[(b)] |
| 4-Methylacetophenone | 122-00-9 | 07.022 | $C_9H_{10}O$ | 134.18 | Solid | 2.10 |
| 4-(4-Methoxyphenyl) butan-2-one | 104-20-1 | 07.029 | $C_{11}H_{14}O_2$ | 178.23 | Liquid | 1.59 |
| 4-Methoxyacetophenone | 100-06-1 | 07.038 | $C_9H_{10}O2$ | 150.18 | Solid | 1.74 |
| 4-(4-Hydroxyphenyl) butan-2-one | 5471-51-2 | 07.055 | $C_{10}H_{12}O_2$ | 164.21 | Solid | 0.94 |
| 1-Phenethyl acetate | 93-92-5 | 09.178 | $C_{10}H_{12}O_2$ | 164.21 | Liquid | 2.28 |

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS no.: EU Flavour Information System numbers.
(a): Logarithm of octanol–water partition coefficient.
(b): Generated from EPI-Suite 4.01.

These substances are produced by chemical synthesis. Several routes of synthesis are available and described in the dossier.[10]

Data were provided on the batch to batch variation for five batches of each additive. The mean content of the active substance for all compounds exceeded the JECFA specifications (Table 2).

Potential contaminants are considered as part of the product specification and are monitored as part of the Hazard Analysis and Critical Control Point system 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.

[9] Racemate.
[10] Technical dossier/Section II.

**Table 2:** Identity of the substances and data on purity

| EU register name | FLAVIS no. | JECFA minimum specification %[a] | Assay % | |
|---|---|---|---|---|
| | | | Average | Range |
| 1-Phenylethan-1-ol | 02.064 | > 99 | 99.3 | 99.1–99.5 |
| Acetophenone | 07.004 | > 98 | 99.2 | 98.5–99.7 |
| Vanillyl acetone | 07.005 | > 95 | 97.7 | 96.7–99.4 |
| 4-Methylacetophenone | 07.022 | > 95 | 97.5 | 95.2–98.3 |
| 4-(4-Methoxyphenyl)butan-2-one | 07.029 | > 96 | 97.5 | 95.9–100 |
| 4-Methoxyacetophenone | 07.038 | > 97[b] | 99.9 | 99.7–100 |
| 4-(4-Hydroxyphenyl)butan-2-one | 07.055 | > 96 | 99.8 | 99.4–100 |
| 1-Phenethyl acetate | 09.178 | > 99 | 99.3 | 99.1–99.5 |

EU: European Union; FLAVIS no.: EU Flavour Information System numbers; JECFA: The Joint FAO/WHO Expert Committee on Food Additives.
(a): FAO (2006).
(b): According to JECFA, minimum assay value is '97 (sum of o,m,p-isomers)' (EFSA, 2008b).

### 3.1.2. Stability

The shelf life for the compounds under assessment ranges from 12 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 eight additives in feed for all animal species without withdrawal. For acetophenone [07.004] and 4-(4-hydroxyphenyl)butan-2-one [07.055], the applicant proposes a normal use level of 5 mg/kg feed and a high use level of 25 mg/kg. For the remaining six additives, the applicant proposes a normal use level of 1 mg/kg feed and a high use level of 5 mg/kg.

## 3.2. Safety

The assessment of safety is based on the highest use level proposed by the applicant (25 mg/kg complete feed for acetophenone and 4-(4-hydroxyphenyl)butan-2-one and 5 mg/kg complete feed for the remaining compounds).

### 3.2.1. Absorption, distribution, metabolism and excretion and residue studies

All compounds belonging to CG 21 are rapidly absorbed from the gastrointestinal tract and share common pathways of metabolism: (i) hydrolysis of esters by carboxylesterases; (ii) reduction of aromatic ketones to alcohols; (iii) oxidation of alcohols to acids; (iv) dealkylation of alkoxyphenols; (v) oxidation of side chains; (vi) ring hydroxylation; and (vii) conjugation of hydroxylated compounds with glucuronic acid and sulfate or acids with glycine (WHO, 2001, 2002).

Acetophenone [07.004] and 1-phenylethan-1-ol [02.064] have been shown to be interconvertible *in vivo*. 1-Phenylethan-1-ol may be excreted in the urine predominantly as the glucuronic acid conjugate. Acetophenone undergoes ω-oxidation to yield α-hydroxyacetophenone. Subsequent stereoselective reduction of the ketone function and oxidation of the terminal alcohol yields mandelic acid, while simple oxidation of the terminal alcohol yields the corresponding keto-acid, which may undergo oxidative decarboxylation to yield benzoic acid, which is excreted as hippuric acid after conjugation with glycine (WHO, 2002).

Studies with related acetophenone derivatives acetovanillone (4-hydroxy-3-methoxy-acetophenone) and paeonol (2-hydroxy-4-methoxyacetophenone) show that substitution of the aromatic ring with hydroxyl groups reduces the reduction rate of the ketone and ω-oxidation of the side chain and favours the conjugation of the aromatic hydroxyl group with sulfate or glucuronic acid. The presence of *O*-methoxy-groups can lead to demethylation and conjugation of the free hydroxyl group. Further hydroxylation with subsequent conjugation of the aromatic ring is also possible (Xie et al., 2008; Gjertsen et al., 1988; Ding et al., 2012).

*p*-Hydroxyderivatives, vanillyl acetone [07.005] and 4-(4-hydroxyphenyl)butan-2-one [07.055] are mainly excreted as glucuronide and sulfate conjugates either unmodified or after reduction to the corresponding alcohol (Scheline, 1991).

When vanillyl acetone (zingerone) was administered to rats (orally or i.p.), 50–56% of the dose was excreted as conjugates of zingerone itself and reduction of the ketone to the corresponding alcohol (zingerol) accounted for an additional 11–13%. Ring hydroxylation to 4(3,4-dihydroxyphenyl)-2-butanone (6%) and side-chain oxidation to 4-hydroxy-3-methoxyphenylacetic acid (8%) were also observed (Monge et al., 1976 as quoted by Scheline, 1991).

Similarly, when 4-(4-hydroxyphenyl)butan-2-one (raspberry ketone) was administered to rats, guinea pigs and rabbits, nearly 90% of the dose was excreted in the urine within 24 h. The main urinary metabolites were *p*-hydroxybenzylacetone and the corresponding alcohol, both mainly conjugated with glucuronic acid and/or sulfate. Oxidative metabolism including ring hydroxylation and side-chain oxidation was observed, the latter pathway leading to 1,2- and 2,3-diol derivatives. Similar pathways were observed in guinea pigs and rabbits (Sporstøl and Scheline, 1982 as quoted by Scheline, 1991).

Studies of metabolism of compounds belonging to CG 21 in target animals are lacking in the scientific literature. Carboxylesterases, responsible for the hydrolysis of esters, are present in the gut, especially of ruminants and liver of several animal species (cattle, pigs, broiler chicks, rabbits and horses), operating the hydrolysis of esters and originating the respective alcohols and acids (Gusson et al., 2006). Aromatic ketones are reduced by carbonyl reductases widely distributed in animal species (Felsted and Bachur, 1980). Oxidation is ubiquitous and phase II conjugation via glucuronidation or sulfation occurs in mammals, although the predominance of one pathway over another varies among animal species (Gupta, 2007). Data collected in a review by Ioannides (2006) show that the cytochrome P450 enzymes responsible for the majority of oxidation reactions are expressed in the liver of the main food-producing animals (cattle, pig, sheep, goat) as well as in the rabbit and chicken (Nebbia et al., 2003). Biotransformation through oxidation followed by conjugation with glucuronic acid and sulfate has also been reported for birds (Pan and Fouts, 1978). The principal cytochrome P450 enzymes responsible for oxidation of xenobiotics, as well as glutathione transferases, are present in the liver of chickens (Blevins et al., 2012). Fish have homologous mechanisms for handling xenobiotic compounds, including both phase 1 and phase 2 biotransformation reactions, and many of the same microsomal and cytosolic enzymes as mammals (Wolf and Wolfe, 2005). Thus, fish can transform endobiotic and xenobiotic compounds through oxidation or hydroxylation, conjugate the metabolites to polar substrates through glucuronide and sulfate conjugation (James and Pritchard, 1987) with further elimination via bile or urine (Di Giulio and Hinton, 2008). Therefore, mammals, fish and birds can also be assumed to have the ability to metabolise and excrete the flavouring substances from CG 21 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

Sub-chronic, repeated-dose studies, with multiple doses tested could be found for 1-phenylethan-1-ol [02.064], acetophenone [07.004], 4-(4-hydroxyphenyl)butan-2-one [07.055] and 1-phenethyl acetate [09.178].

In a 13-week study in rats (males/females, 10 animals/group), 1-phenylethan-1-ol [02.064] dissolved in corn oil was administered by gavage at nominal doses of 0, 93, 187, 375, 750 and 1,500 mg/kg bw per day. Death of 1/10 male rats and 3/10 female rats receiving the highest dose tested were considered treatment related. At the highest dose tested, a reduction of 12% and 7% in final mean body weight was observed in male and female rats, respectively. Rats receiving 750 and 1,500 mg/kg exhibited ataxia, rapid breathing and lethargy up to 30 min after dosing. All effects were reversible. An increase in relative liver weight was observed in male rats in the 375, 750 and 1,500 mg/kg group doses and in female rats at all doses tested. From this study, a low observed adverse effect level (LOAEL) of 93 mg/kg bw per day could be derived (NTP, 1990).

A no observed adverse effect level (NOAEL) of 50 mg/kg bw per day was derived from a 13-week study in rats (15 males/15 females each group), in which 1-phenethyl acetate [09.178] was administered with the diet at nominal doses of 0, 15, 50 and 150 mg/kg bw per day. Decreased leucocyte counts in females and increased relative liver and kidney weights in males were observed at 150 mg/kg bw. There was a transient reduction of red blood cell counts in females at the 50 mg/kg

dose only at the 2 week sampling point. Similarly, the haemoglobin concentration was increased in females given 50 mg/kg at the 6 week sampling point. As these effects were not observed at the higher dose or at other time points or in males, they were considered as transient and not dose related (Gaunt et al., 1974).

The toxicity of acetophenone [07.004] was investigated in a 28-day repeated-dose toxicity study combined with a reproduction/developmental study in rats. The authors of the study identified a NOAEL of 75 mg/kg bw per day for systemic toxicity and a NOAEL of 225 mg/kg bw per day for neurological and reproductive effects. However, as only the summary was available, the FEEDAP Panel is not in a position to confirm these values (Kapp et al., 2003).

The FEEDAP Panel retains the NOAEL of 50 mg/kg bw per day for 1-phenethyl acetate [09.178] and applies the same NOAEL to 1-phenylethan-1-ol [02.064], acetophenone [07.004], 4-methylacetophenone [07.022] and 4-methoxyacetophenone [07.038] on the basis of structure and metabolic similarities.

A NOAEL of 128 mg/kg bw per day was identified in a 13-week study in rats (15 males/15 females each group), in which 4-(4-hydroxyphenyl)butan-2-one [07.055] was administered at dietary doses of 0%, 0.1%, 0.2%, 0.4% and 1.0% (corresponding to 0, 64, 128, 256 and 640 mg/kg bw per day, respectively). Increased relative liver and kidney weights in males were observed in the two higher doses, but these increases were not accompanied by histopathological changes in the organs. A decrease in the body weight in males was observed with the top dose (Gaunt et al., 1970).

The FEEDAP Panel retains the NOAEL of 128 mg/kg bw per day for 4-(4-hydroxyphenyl)butan-2-one [07.055] and applies the same NOAEL to vanillyl acetone [07.005] and 4-(4-methoxyphenyl)-butan-2-one [07.029] on the basis of structure and metabolic similarities.

### 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,b) range from 4.5 to 2,400 μg/person per day, corresponding to 1.0–111.3 μg/kg$^{0.75}$ per day. Table 3 summarises the results of the comparison with human exposure for representative target animals. The body weight of target animals is taken from the default values shown in Table 4.

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

| EU register name | Use level in feed (mg/kg) | Human exposure (μg/kg bw$^{0.75}$ per day)[(a)] | Target animal exposure (μg/kg bw$^{0.75}$ per day) | | |
|---|---|---|---|---|---|
| | | | Salmon | Piglet | Dairy cow |
| 1-Phenylethan-1-ol | 5 | 1.25 | 118 | 526 | 777 |
| Acetophenone | 25 | 0.70 | 588 | 2,632 | 3,885 |
| Vanillyl acetone | 5 | 1.58 | 118 | 526 | 777 |
| 4-Methylacetophenone | 5 | 1.02 | 118 | 526 | 777 |
| 4-(4-Methoxyphenyl)butan-2-one | 5 | 0.21 | 118 | 526 | 777 |
| 4-Methoxyacetophenone | 5 | 6.03 | 118 | 526 | 777 |
| 4-(4-Hydroxyphenyl)butan-2-one | 25 | 111.3 | 588 | 2,632 | 3,885 |
| 1-Phenethyl acetate | 5 | 7.88 | 118 | 526 | 777 |

(a): 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 greatly exceeds that of humans resulting from use in food. As a consequence, safety for the target species at the feed concentration applied cannot be derived from the risk assessment for food use.

As an alternative, the maximum feed concentration considered as safe for the target animal can be derived from the lowest NOAEL available. Toxicological data were available for 1-phenethyl acetate [09.178] and 4-(4-hydroxyphenyl)butan-2-one [07.055], from which a NOAEL value could be derived (see Section 3.2.2). The NOAEL of 50 mg/kg bw per day for 1-phenethyl acetate [09.178] is also applied to 1-phenylethan-1-ol [02.064] and acetophenone [07.004], and its 4-methyl [07.022] and 4-methoxy [07.038] derivatives. Similarly, the NOAEL of 128 mg/kg bw per day for 4-(4-hydroxyphenyl) butan-2-one [07.055] is applied to vanillyl acetone [07.005] and 4-(4-methoxyphenyl)-butan-2-one

[07.029]. 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.

**Table 4:** Maximum safe concentration in feed for different target animals for (**A**) 1-phenylethan-1-ol, 1-phenethyl acetate, acetophenone, 4-methylacetophenone and 4-methoxyacetophenone; (**B**) 4-(4-hydroxyphenyl)butan-2-one, vanillyl acetone and 4-(4-methoxyphenyl)-butan-2-one

| Target animal | Default values | | Maximum safe intake/feed concentration | | | |
|---|---|---|---|---|---|---|
| | Body weight (kg) | Feed intake (g/day)[a] | Intake (mg/day) | | Concentration (mg/kg feed)[b] | |
| | | | A | B | A | B |
| Salmonids | 2 | 40 | 1 | 2.6 | 25 | 64 |
| Veal calves (milk replacer) | 100 | 2,000 | 50 | 128 | 25 | 64 |
| Cattle for fattening | 400 | 8,000 | 200 | 512 | 22 | 56 |
| Dairy cows | 650 | 20,000 | 325 | 832 | 14 | 37 |
| Piglets | 20 | 1,000 | 10 | 26 | 10 | 26 |
| Pigs for fattening | 100 | 3,000 | 50 | 128 | 17 | 43 |
| Sows | 200 | 6,000 | 100 | 256 | 17 | 43 |
| Chickens for fattening | 2 | 120 | 1 | 2.6 | 8 | 21 |
| Laying hens | 2 | 120 | 1 | 2.6 | 8 | 21 |
| Turkeys for fattening | 12 | 400 | 6 | 15 | 15 | 38 |
| Dogs | 15 | 250 | 7.5 | 19 | 26 | 68 |
| Cats[c] | 3 | 60 | 3 | 0.8 | 4.4 | 11 |

(a): 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.
(b): Complete feed containing 88% DM, milk replacer 94.5% DM.
(c): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation (Court and Greenblatt, 1997).

#### 3.2.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that:

- 4-(4-hydroxyphenyl)butan-2-one [07.055] is safe at the proposed maximum use level of 25 mg/kg complete feed for all target species, except chickens for fattening and laying hens and cats, for which the proposed normal use level of 5 mg/kg is considered safe;
- acetophenone [07.004] is safe at the proposed maximum use level of 25 mg/kg complete feed for salmonids, veal calves and dogs, and at the proposed normal use level of 5 mg/kg for the remaining target species (4.4 mg/kg for cats);
- vanillyl acetone [07.005] and 4-(4-methoxyphenyl)-butan-2-one [07.029] are safe at the proposed maximum use level of 5 mg/kg complete feed for all target species; and
- 1-phenylethan-1-ol [02.064], 4-methylacetophenone [07.022], 4-methoxyacetophenone [07.038] and 1-phenethyl acetate [09.178] are safe at the proposed maximum use level of 5 mg/kg complete feed for all target species, except cats for which the proposed normal use level of 1 mg/kg is considered safe.

### 3.2.4. Safety for the consumer

The safety for the consumer of the compounds in CG 21, used as food flavours, has already been assessed by JECFA (WHO, 2001, 2002) and EFSA (2008a,b). All these compounds are presently authorised as food flavourings without limitations.[5] The ADI of 0.1 mg/kg bw per day was established for 1-phenylethan-1-ol [02.064] by JECFA on the basis of the LOAEL of 93 mg/kg bw per day (NTP, 1990) and using an UF of 1,000.

Given the low use levels of CG 21 compounds to be applied in feed, 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 levels of these compounds. Consequently, no safety concern would arise for the consumer from the use of these eight 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[11] 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.

### 3.2.6. Safety for the environment

The additions of naturally occurring substances that will not result in a substantial increase of the concentration in the environment are exempt from further assessment. Examination of the published literature shows that this applies to four substances, namely acetophenone [07.004], 4-methylacetophenone [07.022], 4-(4-methoxyphenyl)-butan-2-one [07.029] and 4-methoxyacetophenone [07.038], which occur in the environment at levels above the application rate of 25 (for acetophenone) 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, 2009).

The other four compounds, namely 1-phenylethan-1-ol [02.064], vanillyl acetone [07.005], 4-(4-hydroxyphenyl)butan-2-one [07.055] and 1-phenethyl acetate [09.178], could not be shown to occur in the environment at levels above the application rate of 5–25 mg/kg feed. For these compounds, 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 Phase I of the EFSA guidance (EFSA, 2008d).

**Table 5:** Predicted environmental concentration (PEC) values of 1-phenylethan-1-ol [02.064], vanillyl acetone [07.005], 4-(4-hydroxyphenyl)butan-2-one [07.055] and 1-phenethyl acetate [09.178] (calculated for lamb manure)

| EU register name | CAS no. | Dose mg/kg | $PEC_{soil}$ (μg/kg) | $PEC_{pore\ water}$ (μg/L) | $PEC_{surface\ water}$ (μg/L) |
|---|---|---|---|---|---|
| 1-Phenylethan-1-ol | 98-85-1 | 5 | 107 | 150 | 50 |
| Vanillyl acetone | 122-48-5 | 5 | 107 | 41 | 14 |
| 4-(4-Hydroxyphenyl)butan-2-one | 5471-51-2 | 25 | 533 | 135 | 45 |
| 1-Phenethyl acetate | 93-92-5 | 5 | 107 | 31 | 10 |

EU: European Union; CAS no.: Chemical Abstracts Service; PEC: predicted environmental concentration.

The $PEC_{soil}$ values are above the threshold of 10 μg/kg (EFSA, 2008d). The PEC for pore water ($PEC_{pore\ water}$), however, is dependent on the sorption, which is different for each compound. For these calculations, the substance-dependent constants such as 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).[12] 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.

The half-life ($DT_{50}$) was calculated using BioWin 3 (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 BioWin4.1 model output.

[11] Technical dossier/Section II/Annex II.3.
[12] Available online: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm

**Table 6:** Physico-chemical properties predicted by EPIWEB 4.1 for 1-phenylethan-1-ol [02.064], vanillyl acetone [07.005], 4-(4-hydroxyphenyl)butan-2-one [07.055] and 1-phenethyl acetate [09.178]

| EU register name | CAS no. | Predicted by EPIWEB 4.1 | | | | |
|---|---|---|---|---|---|---|
| | | $DT_{50}$[(a)] (days) | Molecular weight (g/mol) | Vapour pressure (Pa) | Solubility (mg/L) | $K_{oc}$[(b)] (L/kg) |
| 1-Phenylethan-1-ol | 98-85-1 | 6 | 122.17 | 7.27 | 19,540 | 34 |
| Vanillyl acetone | 122-48-5 | 18 | 194.23 | 0.03 | 3,571 | 142 |
| 4-(4-Hydroxyphenyl) butan-2-one | 5471-51-2 | 13 | 164.21 | 0.10 | 13,460 | 217 |
| 1-Phenethyl acetate | 93-92-5 | 8 | 164.21 | 1.49 | 481 | 187 |

EU: European Union; CAS no.: Chemical Abstracts Service number.
(a): $DT_{50}$, half-life of the additive (EPIWB 4.1.BioWin3).
(b): $K_{oc}$, organic carbon sorption constant (EPIWB 4.1.KocWin2.0).

Calculated predicted exposure for groundwater ($PEC_{pore\ water}$) 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 earthworms, fish, green algae and daphnids from the SMILES notation of the substance. The predicted no effect concentration (PNEC) for terrestrial environment ($PNEC_{soil}$) was determined by dividing the $LC_{50}$ earthworm by a UF of 1,000. The PNEC for 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 soil and aquatic compartments for CG 21 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 / Soil | $LC_{50}$[(a)] earthworm (mg/kg) | | | $PNEC_{soil}$ (μg/kg) | $PEC_{soil}$ (μg/kg) | PEC/ PNEC |
|---|---|---|---|---|---|---|
| 1-Phenylethan-1-ol | – | | | – | 107 | – |
| Vanillyl acetone | 346 | | | 346 | 107 | 0.31 |
| 4-(4-Hydroxyphenyl)butan-2-one | 248 | | | 248 | 533 | 2.15 |
| 1-Phenethyl acetate | 1,479 | | | 1,479 | 107 | 0.07 |
| **Aquatic** | **$LC_{50}$ Fish (mg/L)** | **$LC_{50}$ Daphnids (mg/L)** | **$EC_{50}$[(b)] algae (mg/L)** | **PNEC[(c)] aquatic (μg/L)** | **$PEC_{sw}$[(d)] (μg/L)** | **PEC/ PNEC** |
| 1-Phenylethan-1-ol | 103.55 | 78.95 | 28.34 | 28.34 | 50 | 1.76 |
| Vanillyl acetone | 113.15 | 25.04 | 123.17 | 25.04 | 14 | 0.56 |
| 4-(4-Hydroxyphenyl)butan-2-one | 70.41 | 16.85 | 81.46 | 16.85 | 45 | 2.67 |
| 1-Phenethyl acetate | 11.23 | 22.13 | 8.70 | 8.7 | 10 | 1.15 |

EU: European Union; PNEC: predicted no effect concentration.
(a): $LC_{50}$, the concentration of a test substance which results in 50% mortality of the test species.
(b): $EC_{50}$, the concentration of a test substance which results in 50% of the test animals being adversely affected (i.e. both mortality and sublethal effects).
(c): Experimental data selected in preference to modelled data for derivation of the PNEC.
(d): $PEC_{sw}$: Predicted environmental concentration in surface water.

For 1-phenylethan-1-ol [02.064] it was not possible to derive the $LC_{50}$ for the earthworms using ECOSAR (Table 7), therefore it is not possible to conclude on the safety for the terrestrial compartment. For vanillyl acetone [07.005] and 1-phenethyl acetate [09.178], the ratio PEC/PNEC for soil was < 1, indicating that there is no risk to be expected for the terrestrial environment at the use levels considered safe for target species. For 4-(4-hydroxyphenyl)butan-2-one [07.055], a dose of 25 mg/kg resulted in a PEC/PNEC ratio of 2.149. A dose of 5 mg/kg would result in a $PEC_{soil}$ of 107 μg/kg and in a PEC/PNEC ratio of 0.431.

Concerning the fresh water environment, the $PEC_{sw}$/PNEC ratio for surface water was > 1 for all compounds, except vanillyl acetone for which there is no risk expected to the fresh water environment at the maximum proposed dose (5 mg/kg). For the remaining three compounds, the proposed normal use level (5 mg/kg for 4-(4-hydroxyphenyl)butan-2-one and 1 mg/kg for 1-phenylethan-1-ol and 1-phenetyl acetate) would result in $PEC_{sw}$/PNEC ratio < 1, which is considered safe for this compartment.

The use of all additives in fish feed in land-based aquaculture systems does not give a predicted environmental concentration of the additive (parent compound) in surface water ($PEC_{swaq}$) above the trigger value of 0.1 μg/L when calculated according to the guidance. For sea cages, a dietary concentration of 0.05 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, 2008d).

#### 3.2.6.1. Conclusions on safety for the environment

The proposed maximum use levels in feed for vanillyl acetone [07.005], 4-methylacetophenone [07.022], 4-(4-methoxyphenyl)-butan-2-one [07.029] and 4-methoxyacetophenone [07.038] (5 mg/kg) and for acetophenone [07.004] (25 mg/kg) are unlikely to have detrimental effects on the terrestrial and fresh water environment. For 1-phenylethan-1-ol [02.064], it was not possible to reach a conclusion on the safety for the terrestrial compartment. For 1-phenethyl acetate [09.178], only the normal use level of 1 mg/kg is considered safe. Similarly, only the normal use level of 5 mg/kg is considered safe for 4-(4-hydroxyphenyl)butan-2-one [07.055]. For the marine environment (sea cages), the safe use level is estimated to be 0.05 mg/kg feed for all compounds.

### 3.3. Efficacy

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

## 4. Conclusions

The FEEDAP Panel concludes that 4-(4-hydroxyphenyl)butan-2-one [07.055] is safe at the proposed maximum dose level of 25 mg/kg complete feed for all target species except chickens for fattening and laying hens and cats, for which the proposed normal use level of 5 mg/kg is considered safe; acetophenone [07.004] is safe at the proposed maximum use level of 25 mg/kg complete feed for salmonids, veal calves and dogs, and at the proposed normal use level of 5 mg/kg for the remaining target species (4.4 mg/kg for cats); vanillyl acetone [07.005] and 4-(4-methoxyphenyl)-butan-2-one [07.029] are safe at the proposed maximum dose level of 5 mg/kg complete feed for all target species; 1-phenylethan-1-ol [02.064], 4-methylacetophenone [07.022], 4-methoxyacetophenone [07.038] and 1-phenethyl acetate [09.178] are safe at the proposed maximum dose level of 5 mg/kg complete feed for all target species, except cats for which the proposed use level of 1 mg/kg is considered safe.

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

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

No risk for the environment is expected when the proposed maximum use levels in feed for vanillyl acetone [07.005], 4-methylacetophenone [07.022], 4-(4-methoxyphenyl)-butan-2-one [07.029] and 4-methoxyacetophenone [07.038] (5 mg/kg) and for acetophenone [07.004] (25 mg/kg) are not exceeded. For 1-phenylethan-1-ol [02.064], it was not possible to reach a conclusion on the safety for the terrestrial compartment. For 1-phenethyl acetate [09.178], only the normal use level of 1 mg/kg is considered safe. Similarly, only the normal use level of 5 mg/kg is considered safe for 4-(4-hydroxyphenyl)butan-2-one [07.055].

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 21 – aromatic ketones, secondary alcohols and related esters 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 21 – aromatic ketones, secondary alcohols and related esters for all animal species and categories. May 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
3) Chemically defined flavourings from Flavouring Group 21 – aromatic ketones, secondary alcohols and related esters for all animal species and categories. January 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
4) Chemically defined flavourings from Flavouring Group 21 – aromatic ketones, secondary alcohols and related esters for all animal species and categories. June 2016. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Chemically Defined Flavourings – Group 21 (CDG 21 – Aromatic ketones, secondary alcohols and related esters).
6) Comments from Member States.

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## Abbreviations

| | |
|---|---|
| ADI | acceptable daily intake |
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CD | Commission Decision |

| | |
|---|---|
| CDG | chemically defined group |
| CEF | EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CG | chemical group |
| DM | dry matter |
| $DT_{50}$ | degradation half-time |
| EC | European Commission |
| $EC_{50}$ | half-maximal effective concentration |
| ECOSAR | component program of EPI suite™ |
| 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 |
| IARC | International Agency for Research on Cancer |
| 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 |
| LOAEL | low observed adverse effect level |
| NOAEL | no observed adverse effect level |
| PEC | predicted environmental concentration |
| $PEC_{sed}$ | predicted environmental concentration of the additive (parent compound) in sediment |
| $PEC_{swaq}$ | predicted environmental concentration of the additive (parent compound) in surface water |
| PNEC | predicted no effect concentration |
| SMILES | Simplified Molecular Input Line Entry Specification |
| TNO | the 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 Aromatic ketones, secondary alcohols and related esters

The *Chemically Defined Flavourings - Group 21* (*Aromatic ketones, secondary alcohols and related esters*), in this application comprises ten 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%.

*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 *CDG21* 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 *CDG21* 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 *CDG21* 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 *CDG21* 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.

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*.