SCIENTIFIC OPINION

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Safety and efficacy of aryl-substituted primary alcohol, aldehyde, acid, ester and acetal derivatives belonging to chemical group 22 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 18 compounds belonging to chemical group (CG) 22. They are currently authorised as flavours in food. The FEEDAP Panel concludes that: cinnamaldehyde [05.014] is safe at the maximum use level of 125 mg/kg complete feed for salmonids, veal calves and dogs, and at 25 mg/kg for the remaining target species; cinnamyl alcohol [02.017], 3-phenylpropan-1-ol [02.031], 3-(*p*-cumenyl)-2-methylpropionaldehyde [05.045], α-methylcinnamaldehyde [05.050], 3-phenylpropanal [05.080], cinnamic acid [08.022], cinnamyl acetate [09.018], cinnamyl butyrate [09.053], 3-phenylpropyl isobutyrate [09.428], cinnamyl isovalerate [09.459], cinnamyl isobutyrate [09.470], ethyl cinnamate [09.730], methyl cinnamate [09.740] and isopentyl cinnamate [09.742] are safe at the proposed maximum use level of 5 mg/kg complete feed for all target species; 2-phenylpropanal [05.038], α-pentylcinnamaldehyde [05.040] and α-hexylcinnamaldehyde [05.041] are safe at the proposed maximum dose level of 5 mg/kg complete feed for all target species except cats, for which 1 mg/kg is safe. No safety concern would arise for the consumer from the use of these compounds up to the highest proposed level in feeds. Irritation and sensitisation hazards for skin and irritation for eye are recognised for the majority of the compounds under application. Respiratory exposure may also be hazardous. For the majority of the compounds belonging to CG 22, the maximum proposed use levels are considered safe for the environment. For α-pentylcinnamaldehyde and α-hexylcinnamaldehyde, a use level up to 0.1 mg/kg feed would not cause a risk for the terrestrial and fresh water compartments. 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.

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Keywords: sensory additives, aryl derivatives, primary alcohols, related esters, safety, chemical group 22

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Table of contents

1. Introduction

1.1. Background and Terms of Reference

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

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation of 19 substances belonging to chemical group (CG) 22 (cinnamyl alcohol, 3-phenylpropan-1-ol, cinnamaldehyde, 2-phenylpropanal, α-pentylcinnamaldehyde, α-hexylcinnamaldehyde, 3-(*p*-cumenyl)-2-methylpropionaldehyde, α-methyl-cinnamaldehyde, 3-phenylpropanal, 5-methyl-2-phenylhex-2-enal, cinnamic acid, cinnamyl acetate, cinnamyl butyrate, 3-phenylpropyl isobutyrate, cinnamyl isovalerate, cinnamyl isobutyrate, ethyl cinnamate, methyl cinnamate and isopentyl cinnamate), when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). CG 22 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[3] as 'aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones'.

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 cinnamyl alcohol [The EU Flavour Information System (FLAVIS) Number 02.017], 3-phenylpropan-1-ol [02.031], cinnamaldehyde [05.014], 2-phenylpropanal [05.038], α-pentylcinnamaldehyde [05.040], α-hexylcinnamaldehyde [05.041], 3-(*p*-cumenyl)-2-methyl-propionaldehyde [05.045], α-methylcinnamaldehyde [05.050], 3-phenylpropanal [05.080], 5-methyl-2-phenylhex-2-enal [05.099], cinnamic acid [08.022], cinnamyl acetate [09.018], cinnamyl butyrate [09.053], 3-phenylpropyl isobutyrate [09.428], cinnamyl isovalerate [09.459], cinnamyl isobutyrate [09.470], ethyl cinnamate [09.730], methyl cinnamate [09.740] and isopentyl cinnamate [09.742] when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

All 19 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, 2005) and were considered safe for use in food. No acceptable daily intake values were established.

Subsequently, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) assessed the same compounds and concluded that 18 out of the 19 compounds under application do not give rise to safety concerns when used as flavour in food (EFSA, 2008a,b, 2009a) but raised a concern for genotoxicity for 5-methyl-2-phenylhex-2-enal [05.099] and requested additional genotoxicity

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2] On 13/03/2013, EFSA was informed by the applicant that FFAC EEIG was liquidated on 19/12/2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures). Avenue Louise 130A, Box 1, 1050 Brussels, Belgium.

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

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

data (EFSA, 2008a; EFSA CEF Panel, 2013). Consequently, the FEEDAP Panel will not proceed with an assessment of this compound until the outstanding issue has been addressed.

The remaining 18 compounds are currently listed in the European Union (EU) database of flavouring substances[5] and in the EU Register of Feed Additives, and thus authorised for use in food and feed in the EU. They have not been previously assessed by EFSA as feed additives.

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

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[7] in support of the authorisation request for the use of the compounds belonging to CG 22 as feed additives. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008 and the applicable EFSA guidance documents.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has sought to use the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of flavourings of the 'aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones' 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 'aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones' is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008c), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

3.1. Characterisation

3.1.1. Characterisation of the flavouring additives

The molecular structures of the 18 additives under application are shown in Figure 1 and their physicochemical characteristics in Table 1.

These substances are produced by chemical synthesis. Typical routes of synthesis available in the literature are described in the dossier.[9]

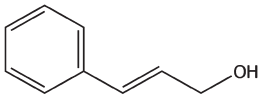
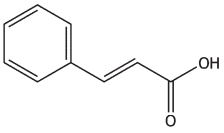
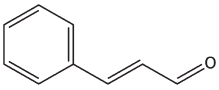
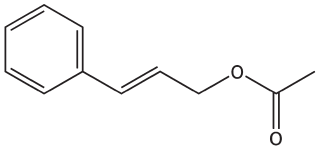
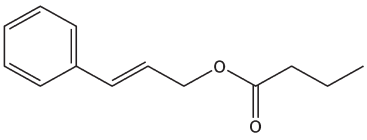
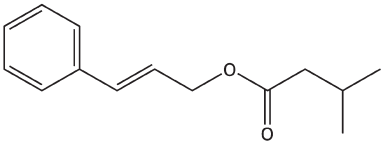
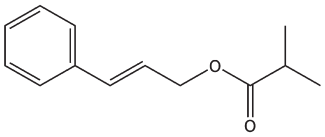
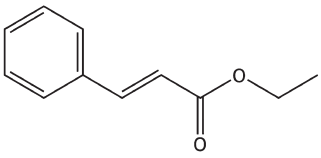
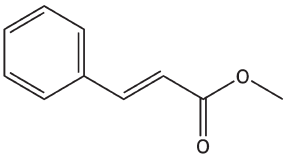
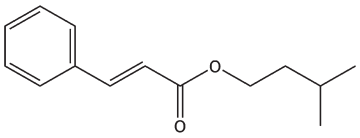
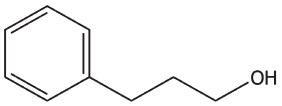
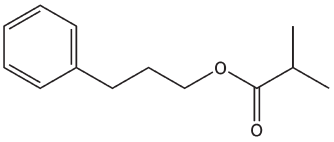
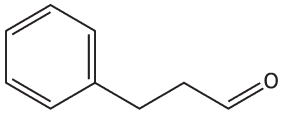
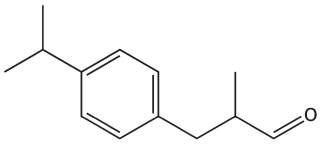
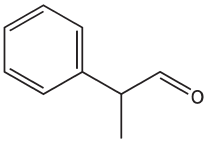
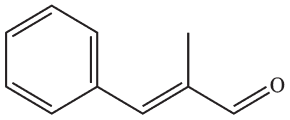
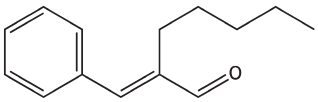
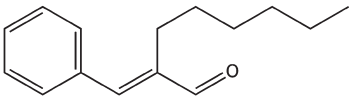
[5] Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the 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-2010-0076.

[8] The full report is available on the EURL website https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0076.pdf

[9] Technical dossier/Section II.

Cinnamyl alcohol [02.017]* 	Cinnamic acid [08.022]* 	Cinnamaldehyde [05.014]*, (a) 
Cinnamyl acetate [09.018]* 	Cinnamyl butyrate [09.053]* 	Cinnamyl isovalerate [09.459]* 
Cinnamyl isobutyrate [09.470]* 	Ethyl cinnamate [09.730]* 	Methyl cinnamate [09.740]* 
Isopentyl cinnamate [09.742]* 	3-Phenylpropan-1-ol [02.031] 	3-Phenylpropyl isobutyrate [09.428] 
3-Phenylpropanal [05.080] 	3-(*p*-Cumenyl)-2-methyl propionaldehyde [05.045]** 	2-Phenylpropanal [05.038]** 
α-Methylcinnamaldehyde [05.050]* 	α-Pentylcinnamaldehyde [05.040]* 	α-Hexylcinnamaldehyde [05.041]* 

*: (*E*)-isomer shown. Composition of the stereoisomeric mixture to be specified.
(a): The configuration of the double bond in cinnamaldehyde [05.014] has not been specified. However, the substance is anticipated to contain more than 97% *trans*-cinnamaldehyde (EFSA, 2009b).
**: racemate.

Figure 1: Molecular structures and [FLAVIS numbers] of the 18 flavouring compounds under assessment

Table 1: Chemical Abstracts Service (CAS) and FLAVIS numbers and some characteristics of the 18 flavouring compounds under assessment

EU Register name	CAS No	FLAVIS No	Molecular formula	Molecular weight	Physical state	Log K_{ow}[(a)]
Cinnamyl alcohol	104-54-1	02.017	$C_9H_{10}O$	134.18	Solid	1.95
3-Phenylpropan-1-ol	122-97-4	02.031	$C_9H_{12}O$	136.19	Liquid	1.88
Cinnamaldehyde	104-55-2	05.014	C_9H_8O	132.16	Liquid	1.9
2-Phenylpropanal	93-53-8	05.038	$C_9H_{10}O$	134.18	Liquid	1.96
α-Pentylcinnamaldehyde	122-40-7	05.040	$C_{14}H_{18}O$	202.30	Liquid	4.33
α-Hexylcinnamaldehyde	101-86-0	05.041	$C_{15}H_{20}O$	216.32	Liquid	4.8*
3-(*p*-Cumenyl)-2-ethylpropionaldehyde	103-95-7	05.045	$C_{13}H_{18}O$	190.29	Liquid	3.9
α-Methylcinnamaldehyde	101-39-3	05.050	$C_{10}H_{10}O$	146.19	Liquid	2.68
3-Phenylpropanal	104-53-0	05.080	$C_9H_{10}O$	134.18	Liquid	2.03
Cinnamic acid	621-82-9	08.022	$C_9H_8O_2$	148.16	Solid	2.13
Cinnamyl acetate	103-54-8	09.018	$C_{11}H_{12}O_2$	176.22	Liquid	2.85
Cinnamyl butyrate	103-61-7	09.053	$C_{13}H_{16}O_2$	204.27	Liquid	3.83
3-Phenylpropyl isobutyrate	103-58-2	09.428	$C_{13}H_{18}O_2$	206.28	Liquid	3.97
Cinnamyl isovalerate	140-27-2	09.459	$C_{14}H_{18}O_2$	218.30	Liquid	4.25
Cinnamyl isobutyrate	103-59-3	09.470	$C_{13}H_{16}O_2$	204.27	Liquid	3.76
Ethyl cinnamate	103-36-6	09.730	$C_{11}H_{12}O_2$	176.22	Liquid	2.99
Methyl cinnamate	103-26-4	09.740	$C_{10}H_{10}O_2$	162.19	Solid	2.62
Isopentyl cinnamate	7779-65-9	09.742	$C_{14}H_{18}O_2$	218.30	Liquid	4.25*

EU: European Union; CAS No: Chemical Abstract Service number; Flavis No: EU Flavour Information System number.
(a): Logarithm of octanol–water partition coefficient.
*: Calculated from EPIWEB 4.1

Batch-to-batch variation data were provided for five batches of each additive except 2-phenylpropanal [05.038], for which only one batch was available due to the low use volume.[10] The content of the active substance for all compounds exceeded the JECFA specifications (Table 2).

Table 2: Identity of the substances and data on purity

EU Register name	FLAVIS No	JECFA specification minimum %[(a)]	Assay %	
			Average	Range
Cinnamyl alcohol	02.017	98	99.1	98.2–99.5
3-Phenylpropan-1-ol	02.031	98	98.6	98.0–99.2
Cinnamaldehyde	05.014	98	99.0	98.0–99.4
2-Phenylpropanal	05.038	95	98.7[(b)]	–
α-Pentylcinnamaldehyde	05.040	97	98.2	97.3–99.2
α-Hexylcinnamaldehyde	05.041	95	98.0	97.8–98.3
3-(*p*-Cumenyl)-2-ethylpropionaldehyde	05.045	90	97.5	90.3–99.6
α-Methylcinnamaldehyde	05.050	95	97.8	95.6–99.2
3-Phenylpropanal	05.080	95	97.1	95.3–99.4
Cinnamic acid	08.022	98	99.9	99.1–100.4
Cinnamyl acetate	09.018	98	99.3	98.8–99.6
Cinnamyl butyrate	09.053	98	98.3	97.7–98.7
3-Phenylpropyl isobutyrate	09.428	98	99.2	98.0–99.7
Cinnamyl isovalerate	09.459	95	98.7	97.6–99.9
Cinnamyl isobutyrate	09.470	96	97.8	97.0–98.2

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

EU Register name	FLAVIS No	JECFA specification minimum %[a]	Assay %	
			Average	Range
Ethyl cinnamate	09.730	99	99.1	98.8–99.4
Methyl cinnamate	09.740	98	99.8	99.2–100
Isopentyl cinnamate	09.742	97	99.6	99.0–100

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

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

3.1.2. Stability

The shelf-life for the compounds under assessment ranges from 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 18 additives in feed for all animal species without withdrawal. For cinnamaldehyde [05.014], the applicant proposes a normal use level of 25 mg/kg feed and a high use level of 125 mg/kg. For the remaining 17 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 (125 mg/kg complete feed for cinnamaldehyde and 5 mg/kg complete feed for the remaining compounds).

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

3.2.1.1. Uptake, distribution and excretion

[^{14}C]-Radiolabelled cinnamyl alcohol, cinnamaldehyde and cinnamic acid (335, 330 and 370 mg/kg body weight (bw), respectively) were individually applied by gavage to male Fischer 344 rats. Between 77% and 83% of the radioactivity was excreted in the urine within 24 h, and 0.9–16% occurred in the faeces. More than 90% of the administered dose of any of the three substances was recovered in the urine and faeces within 72 h. Administration of the same compounds to groups of CD-1 mice by intraperitoneal (i.p.) injection resulted in a similar pattern of excretion in the urine and faeces at 24 h (75–93%) and 72 h (> 93%) (Nutley, 1990; as quoted by WHO, 2001b).

The tissue distribution and excretion of cinnamaldehyde were studied in male Fischer 344 rats pretreated with single daily oral doses of 5, 50 or 500 mg/kg bw of cinnamaldehyde by gavage for 7 days and the same single oral dose of [^{14}C]-cinnamaldehyde 24 h later. Further groups received no pretreatment but the same single doses. After 24 h, > 80% of the radiolabel was recovered in the urine and < 7% in the faeces from all rats, regardless of dose; after 72 h, the recovery in the urine and faeces was about 90–95%. The radioactivity level in blood was less than 0.15% of the dose after 24 h for all doses tested. The radiolabel was distributed primarily to the gastrointestinal tract, kidneys and liver in all groups. A small amount of the dose was distributed to fat (0.2–0.9%) and < 0.3% cumulatively to the brain, heart, lung, spleen, and testes. The elimination half-life for [^{14}C] was 5–9 h for the whole blood and liver, 5–8 h for muscle, and was considerably longer for fat tissue, ranging from 17.3 h at 5 mg/kg to 73 h at 500 mg/kg. Radiolabel was still detectable in the fat of animals killed 3 days after receiving 50 or 500 mg cinnamaldehyde/kg bw (Sapienza et al., 1993). In another study, disposition and excretion of [^{14}C]-cinnamaldehyde administered to Fischer 344 rats and CD-1 mice (2 or 250 mg/kg bw) were not sex- and dose-related (Peters and Caldwell, 1994).

To investigate the effect of dose on the disposition of cinnamic acid, five doses in the range 0.08–400 mg/kg bw of [^{14}C]-cinnamic acid were administered orally to male Fischer 344 rats or by

i.p. injection to male CD-1 mice. Excretion of radiolabel was essentially the same in both species and was not influenced by dose size. After 24 h, the recovery in the urine was 73–88% for rats and 78–93% for mice; after 72 h, 85–100% of the radiolabel was recovered from rats and 89–100% from mice, mainly in the urine. Only trace amounts of radiolabel were present in the carcass (< 1%), indicating that cinnamic acid was readily and quantitatively excreted at all doses (Caldwell and Nutley, 1986).

Cinnamic alcohol, cinnamaldehyde and cinnamic acid therefore appear to undergo rapid absorption and excretion, independently of the dose up to 250 mg/kg bw, species, sex and mode of administration.

The pharmacokinetic of cinnamic acid was also studied in human volunteers. Eleven individuals each received a single intravenous dose of cinnamic acid equivalent to 5 mg/kg bw. Analysis of blood showed that none of the administered dose was detectable after 20 min (WHO, 2001b).

Rapid absorption and excretion was demonstrated also for the saturated 3-phenylpropionic acid in humans (Pollit, 1974).

3.2.1.2. Metabolism

Generally, the enzymatic pathways involved in the metabolism of CG 22 compounds are: (i) hydrolysis of esters by carboxylesterases, producing the alcohol (cinnamyl or analogue) and the corresponding acid, (ii) oxidation of alcohols and aldehydes to acids, (iii) β-oxidation of the side chain of acids, leading to benzoic acid (iv) conjugation of acids with glucuronic acid or conjugation of aldehydes with glutathione (minor pathways), and (v) conversion of cinnamic acid to acyl CoA esters with subsequent conjugation with amino acids and elimination in urine (WHO, 2001b; EFSA, 2008b).

Hydrolysis of esters

An oral dose of 240 mg/kg bw methyl cinnamate was rapidly and almost completely (95%) absorbed from the gut of rats. It was partially hydrolysed to cinnamic acid in the stomach (9%) and subsequently in the intestinal wall. The rate of absorption of cinnamic acid and methyl cinnamate from the gut was similar. No ester was detected in the peripheral blood of dosed rabbits or rats. Only traces were detected in portal and heart blood taken from the rats, indicating that almost complete hydrolysis of methyl cinnamate had occurred during intestinal absorption. The ability of intestinal esterases to hydrolyse methyl cinnamate was also demonstrated *in vitro* (Fahelbum and James, 1977). Ethyl cinnamate administered subcutaneously to a cat also produced only cinnamic acid metabolites in the urine (Dakin, 1909). The other esters present in this opinion are also likely to be hydrolysed and thus transformed to the respective alcohols and acids.

Oxidative- and phase II metabolism

When cinnamyl alcohol was administered orally to four rats at a dose of 335 mg/kg bw, 52% was recovered in the urine within 24 h as hippuric acid. Ten minor metabolites cumulatively accounted for about 10% of the dose. In mice, hippuric acid was the major urinary metabolite of cinnamyl alcohol when administered by i.p. injection (Nutley, 1990 as quoted by WHO, 2001b).

To investigate the effects of sex, dose size and route of administration on the excretion pattern and metabolic profile, *trans*-[^{14}C]cinnamaldehyde was given at doses of 2 and 250 mg/kg bw by i.p. injection to male and female Fischer 344 rats and CD-1 mice, and at doses of 250 mg/kg bw by oral gavage to male rats and mice only. In both species, and independently of sex, dose size and administration route, the major urinary metabolites were formed from oxidation of cinnamaldehyde to cinnamic acid, which was subsequently oxidised to benzoic acid. The major urinary metabolite was hippuric acid (71–75% in mice and 73–87% in rats). Small amounts of 3-hydroxy-3-phenylpropionic acid (0.4–4%), benzoic acid (0.4–3%) and benzoyl glucuronide (0.8–7.0%) were also detected. Cinnamic acid was excreted as glycine conjugate (4–13%) in mice only. In both species, approximately 6–9% of the dose was excreted within 24 h as glutathione conjugates of cinnamaldehyde (Peters and Caldwell, 1994).

In another study examining specifically the metabolites produced by conjugation of cinnamaldehyde with glutathione, approximately 15% of a dose of 250 mg/kg bw administered to rats by gavage was excreted in the urine as mercapturic acid derivatives. Two sulfur-containing metabolites were isolated from rat urine and identified as *N*-acetyl-*S*–(1-phenyl-3-hydroxypropyl)-cysteine and *N*-acetyl-*S*–(1-phenyl-2-carboxyethyl)-cysteine in a 4:1 ratio. The hydroxypropyl mercapturate was also isolated from the urine of rats administered with cinnamyl alcohol (125 mg/kg bw) and accounted for 9% of the dose (Delbressine et al., 1981).

[^{14}C]-Cinnamic acid (six doses in the range 0.08–400 mg/kg bw) were administered orally to male Fischer 344 rats or by i.p. injection to male CD-1 mice. The metabolites identified at all doses included the major metabolite hippuric acid (44–77%), benzoyl glucuronide, 3-hydroxy-3-phenylpropionic acid, benzoic acid and unchanged cinnamic acid. Acetophenone and cinnamoylglycine were detected only in the urine of mice. As the dose size increased (> 80 mg/kg bw), the percentage of hippuric acid decreased in rat urine while the percentages of benzoyl glucuronide (0.5–5%) and benzoic acid (0.4–2%) increased, suggesting that some limitation of the glycine conjugation pathway occurs at these doses. The fact that the excretion of 3-hydroxy-3-phenylpropionic acid differed little over the dose range (0.2–0.9%) supports the conclusion that the capacity of the β-oxidation pathway is not limited at doses of cinnamic acid up to 400 mg/kg bw in male rats. At all doses, mice excreted only a small proportion of benzoyl glucuronide, indicating that this conjugation reaction is of minimal importance in this species (Nutley et al., 1994).

After administration of a single oral dose of ring-deuterated 3-phenylpropionic acid to one individual, the total amount of administered deuterobenzoic acid was isolated from alkaline hydrolysed urine within 100 min (Pollit, 1974). This finding shows that the side chain of the saturated counterpart of cinnamic acid is also degraded to the corresponding benzoic acid, which is further metabolised mainly to hippuric acid.

Branched side chains of cinnamyl derivatives can alter the oxidative metabolism. Compounds containing α-methyl substituents are extensively metabolised via β-oxidation, yielding mainly the corresponding hippuric acid derivative. When β-oxidation is inhibited to some extent by the presence of larger substituents located at the α- or β-position, the carboxylic acid may be directly conjugated with glucuronic acid and excreted (WHO, 2001b). A benzoic acid metabolite was isolated from the urine of dogs given either α-methylcinnamic acid or α-methylphenylpropionic acid (Kay and Raper, 1924 as quoted by WHO, 2001b). While α-methylcinnamic acid undergoes oxidation to benzoic acid, α-ethyl- and α-propylcinnamic acids are excreted as such (Carter, 1941 as quoted by WHO, 2001b). α-Ethylcinnamic alcohol and α-ethylcinnamaldehyde administered orally to rabbits resulted in urinary excretion of α-ethylcinnamic acid and of small amounts of benzoic acid (Fischer & Bielig, 1940 as quoted by WHO, 2001b). These observations suggest that α-methylcinnamaldehyde undergoes oxidation to benzoic acid, while higher homologues may be excreted primarily unchanged or as the conjugated form of the cinnamic acid derivative.

Controlled ADME studies of CG 22 compounds in target species are not available. Out of the 18 compounds under assessment, eight are esters and are expected to be hydrolysed. Carboxylesterases, responsible for the hydrolysis of esters, are present in the gut, especially of ruminants, and the 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). Carboxylesterase activity also plays a significant role in detoxification processes in fish (Li and Fan, 1997; Di Giulio and Hinton, 2008). After hydrolysis, the metabolism of the compounds under assessment can be represented by the fate of cinnamic acid and its analogue 3-phenylpropionic acid resulting from oxidation of the precursors alcohol and aldehydes and hydrolysis of esters. Carboxylic acid:CoA ligases that convert these acids to the respective CoA esters (the first step to proceed to β-oxidation and amino acid conjugation) were shown to be expressed in the liver and kidney of ruminants (Vessey and Hu, 1995), the gut of pigs (Vessey, 2001), and in the liver and kidney of fish (Schlenk et al., 2008). In ruminants, the metabolism of these compounds largely starts in the rumen. When cinnamic acid was infused in the rumen or abomasum of ruminants, 70% was recovered in the urine as benzoic acid conjugates (Martin, 1982a). In the rumen, 3-phenylpropionic acid originated by microbial metabolism of hydroxycinnamic acids, is absorbed and oxidised in organism and eliminated as benzoic acid in urine (Martin, 1982b). Also in sheep, Pagella et al., 1997; showed that 3-phenylpropionic acid (oxidation product of the two CG 22 compounds 3-phenylpropanol and 3-phenylpropanal) infused in the rumen was excreted in the urine mainly as hippuric acid. Conjugation of carboxylic acids with amino acids exhibits some species specificity. After oral administration of 50 mg/kg of radiolabelled benzoate to several animal species, rabbit, pig, cat, and dog eliminated almost all the initial dose in the urine after 24 h as hippuric acid. In the dog, approximately 20% was excreted as benzoyl glucuronide (Bridges et al., 1970). Many other target species can also form glucuronides, although this is generally a minor route of excretion. Several types of birds, including chickens, excrete benzoic acid as ornithuric acid (Baldwin et al., 1960; Letizia et al., 2005). In fish, benzoic acid is conjugated mainly with taurine (Schlenk et al., 2008). Although at a minor rate, glucuronide derivatives can be formed and conjugation pathway with glucuronic acid can also be carry out by all target species (Watkins and Klaassen, 1986; James, 1987; Gusson et al., 2006). Therefore, mammals, fish and birds, have the

ability to metabolise and excrete the flavouring substances from CG 22, and there is no evidence that they or their metabolites would accumulate in tissues. The FEEDAP Panel notes that for feline species the capacity for conjugation is limited (Shrestha et al., 2011; Court, 2013).

3.2.2. Toxicological studies

Subchronic, repeated-dose studies, with multiple doses tested could be found for cinnamaldehyde [05.014], 2-phenylpropanal [05.038] and α-pentylcinnamaldehyde [05.040]. Secondary references referred to additional studies with α-methylcinnamaldehyde [05.050] (multiple dose tested).

In a 13-week study, the potential toxicity of *trans*-cinnamaldehyde [05.014] was investigated in Fischer 344/N rats. Groups of 10 male and 10 female rats were given diets containing 0%, 1.25%, 2.5%, 5.0% or 10% microencapsulated *trans*-cinnamaldehyde, corresponding to 0, 620, 1,250, 2,500 or 5,000 mg/kg bw per day. There were no early deaths and no treatment-related clinical toxicity. Average body weights of animals at the three higher doses were decreased compared to controls. The food consumption of treated animals was depressed during the first week, possibly because of unpalatability. No effects were seen on haematological parameters. A treatment-related increase in bile salt concentration and alanine transaminase (ALAT) activity (in males and females at the highest dose) suggested mild cholestasis. Necropsies were performed on all survivors, and tissues from animals at the two highest doses and the control group were examined histologically. Microscopic examination showed no morphological alterations to the liver. Gross and microscopic examination of the stomach and forestomach indicated irritation at all doses of *trans*-cinnamaldehyde. From this study, a no observed effect level (NOEL) of 620 mg/kg bw per day was derived. However, this NTP study is unpublished and is available only in summary form as described in JECFA (WHO, 2001b).

In a second NTP report (2004), *trans*-cinnamaldehyde was tested in a repeated-dose subchronic study of 14 weeks and in a 2-year carcinogenicity study in both rats and mice. In the subchronic study, 20 male and female F344/N rat and B6C3F1 mice were fed microencapsulated *trans*-cinnamaldehyde for 14 weeks. The daily doses were approximately 650, 1,320, 2,550, and 5,475 mg/kg bw per day for male mice and 625, 1,380, 2,680, and 5,200 mg/kg bw per day for female mice. The corresponding doses for rats were approximately 275, 625, 1,300, and 4,000 mg/kg bw per day for males and 300, 570, 1,090 and 3,100 mg/kg bw per day for females. Another 20 rats and mice received untreated feed (untreated controls) or feed containing placebo microcapsules (vehicle controls). A no observed adverse effect level (NOAEL) of 625 mg/kg bw, the lowest dose tested in mice, was derived based on olfactory epithelial degeneration of the nasal cavity in mice given higher doses. From the rat study, a NOAEL of 275 mg/kg bw can be derived based on a treatment-related decrease in ALAT and serum albumin in females and multifocal to diffuse white nodules of the forestomach mucosa in males and females exposed to higher doses. In the 2-year feeding study, groups of 50 male and 50 female F344/N rat and B6C3F1 were fed diets containing microencapsulated *trans*-cinnamaldehyde at doses of 50, 100 or 200 mg/kg bw per day for male and female rats and 125, 270 or 540 (males) or 570 (females) mg/kg bw per day for mice. Under the conditions of the study, there was no evidence of carcinogenic activity of *trans*-cinnamaldehyde in male or female F344/N rats and in male or female B6C3F1 mice (NTP, 2004).

In a subchronic study with Osborne–Mendel rats, groups of 10 males and 10 females were maintained on a diet containing cinnamaldehyde (isomer not specified) at a concentration of 0 (control), 1,000, 2,500 or 10,000 mg/kg, equivalent to 50, 120 and 500 mg/kg bw per day, for 16 weeks. No differences were observed on body weight, food intake, haematological parameters, organ weights and gross pathology. Histological examination of three to four male and female animals at the high dose revealed slight hepatic cellular swelling and slight hyperkeratosis of the squamous epithelium of the stomach. The NOEL was therefore 120 mg/kg bw per day (Hagan et al., 1967; not available, quoted by JECFA, FAS46). This experiment confirms the findings of the NTP study which showed that the NOAEL is < 500 mg/kg bw. The FEEDAP Panel retains the NOAEL of 275 mg/kg bw per day derived from the 14-week study with *trans*-cinnamaldehyde (NTP, 2004) as a group NOAEL for cinnamaldehyde and related cinnamyl derivatives.

2-Phenylpropanal [05.038] was administered to rats (males/females, 15 animals/group) by gavage at doses of 0, 10, 50 and 500 mg/kg bw per day for 15-weeks (Pelling et al., 1976). No differences were observed on body weight gain, feed consumption and of the renal function. At the highest dose tested, an increase in the relative weight of several organs (liver, kidney, stomach, pituitary gland in both sexes, heart and spleen in females only) was observed. These changes were not associated with

histopathological changes. From this study, a NOAEL of 50 mg/kg bw per day could be derived based on increased relative organ weights at the highest dose level.

In a 14-week study in rats (males/females, 15 animals/group), α-pentylcinnamaldehyde [05.040] was administered with the diet at doses of 0, 80, 400 and 4,000 mg/kg feed (equivalent to intakes of 6.1/6.7, 29.9/34.9 and 287.3/320.3 (males/females) mg/kg bw per day). An increase in the relative liver and kidney weight was observed at the highest dose tested, but they were not associated with any histopathological changes. The NOAEL derived from this study is 30 mg/kg bw per day (Carpanini et al., 1973).

Secondary references refer to a 90-day study in rats (five males/five females) fed α-methylcinnamaldehyde [05.050] at doses of 0, 58, 120 or 220 mg/kg bw for 90 days. Growth and food intake were recorded weekly, as were the results of regular examinations for physical appearance, behaviour and efficiency of food use. At week 12, urine samples were collected and analysed for the presence of sugar and albumin, and blood samples were taken for determination of haemoglobin. No statistically significant differences were found between treated and control animals, and no differences in the liver or kidney weights were seen. Thus, the NOAEL for this study is 220 mg/kg bw, the highest dose applied. (Trubeck Laboratories, 1958c, as described by WHO, 2001b). Although the study report was not available, the NOAEL of 220 mg/kg bw is supported by the NOAEL of 275 mg/kg bw per day taken for cinnamaldehyde and related compounds.

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, 2009a,b) ranges from 2.4 to 2,400 μg/person per day, corresponding to 0.11–112.3 μg/kg$^{0.75}$ per day. Table 3 summarises the result of the comparison with human exposure for representative target animals. The body weight of target animals is taken from the default values shown in Table 4.

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

EU Register name	Use level in feed (mg/kg)	Human exposure (μg/kg bw$^{0.75}$ per day)[(a)]	Target animal exposure (μg/kg bw$^{0.75}$ per day)		
			Salmon	Piglet	Dairy cow
Cinnamyl alcohol	5	69.6	118	526	777
3-Phenylpropan-1-ol	5	2.37	118	526	777
Cinnamaldehyde	125	97.4	2,941	13,158	19,425
2-Phenylpropanal	5	5.10	118	526	777
α-Pentylcinnamaldehyde	5	1.02	118	526	777
α-Hexylcinnamaldehyde	5	3.43	118	526	777
3-(*p*-Cumenyl)-2-ethylpropionaldehyde	5	0.83	118	526	777
α-Methylcinnamaldehyde	5	0.11	118	526	777
3-Phenylpropanal	5	0.74	118	526	777
Cinnamic acid	5	1.30	118	526	777
Cinnamyl acetate	5	8.35	118	526	777
Cinnamyl butyrate	5	0.12	118	526	777
3-Phenylpropyl isobutyrate	5	0.17	118	526	777
Cinnamyl isovalerate	5	0.18	118	526	777
Cinnamyl isobutyrate	5	0.51	118	526	777
Ethyl cinnamate	5	4.13	118	526	777
Methyl cinnamate	5	111.3	118	526	777
Isopentyl cinnamate	5	0.32	118	526	777

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

As an alternative, the maximum feed concentration considered as safe for the target animal can be derived from the lowest NOAEL available. Toxicological data, from which a NOAEL value could be derived, were available for cinnamaldehyde [05.014] (275 mg/kg bw), 2-phenylpropanal [05.038] (50 mg/kg bw), α-pentylcinnamaldehyde [05.040] (30 mg/kg) and α-methylcinnamaldehyde [05.050] (220 mg/kg bw). The FEEDAP Panel considers a NOAEL of 275 mg/kg bw per day derived for cinnamaldehyde [05.014] for all cinnamyl derivatives, i.e. cinnamyl alcohol [02.017], cinnamic acid [08.022], cinnamyl acetate [09.018], cinnamyl butyrate [09.053], cinnamyl isovalerate [09.459], cinnamyl isobutyrate [09.470], ethyl cinnamate [09.730], methyl cinnamate [09.740] and isopentyl cinnamate [09.742]. The same NOAEL is applied to 3-phenylpropan-1-ol [02.031] and its ester 3-phenylpropyl isobutyrate [09.428] on the basis of structure and metabolic similarities. The NOAEL of cinnamaldehyde is also applied to 3-phenylpropanal [05.080] with an additional uncertainty factor (UF) of 2 to take account of a presumed greater reactivity compared to cinnamaldehyde because of the absence of conjugation between the aldehyde function and the aromatic ring. This extrapolation is extended to 3-(*p*-cumenyl)-2-methylpropionaldehyde [05.045]. Similarly, the NOAEL of 30 mg/kg bw per day for α-pentylcinnamaldehyde [05.040] is applied to α-hexylcinnamaldehyde [05.041]. For α-methylcinnamaldehyde [05.050], the NOAEL is set to 220 mg/kg bw per day.

Applying an UF of 100 to the respective NOAELs, the maximum safe intake for the target species was derived for the 18 compounds following the EFSA Guidance for sensory additives (EFSA FEEDAP Panel, 2012a), and thus the maximum safe feed concentration was calculated (Tables 4 and 5). For 3-phenylpropanal [05.080] and 3-(*p*-cumenyl)-2-methylpropionaldehyde [05.045], the UF is increased by a factor of 2 because of presumed greater reactivity compared to cinnamaldehyde. The UF for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation and glycine conjugation (Court and Greenblatt, 1997).

Table 4: Maximum safe concentration in feed for different target animals for (**A**) cinnamaldehyde and 11 structurally related compounds and (**B**) for 3-phenylpropanal and 3-(*p*-cumenyl)-2-methylpropionaldehyde, derived using a NOAEL 275 mg/kg bw and applying an UF of 100 (**A**) and 200 (**B**)

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	5.5	2.8	138	69
Veal calves (milk replacer)	100	2,000	275	138	138	69
Cattle for fattening	400	8,000	1,100	550	121	61
Dairy cows	650	20,000	1,788	894	79	39
Piglets	20	1,000	55	28	55	28
Pigs for fattening	100	3,000	275	138	92	46
Sows	200	6,000	550	275	92	46
Chickens for fattening	2	120	5.5	2.8	46	23
Laying hens	2	120	5.5	2.8	46	23
Turkeys for fattening	12	400	33	17	83	41
Dogs	15	250	41	21	145	73
Cats[c]	3	60	1.7	0.8	24	12

A: cinnamaldehyde, cinnamyl alcohol, 3-phenylpropan-1-ol, cinnamic acid, cinnamyl acetate, cinnamyl butyrate, 3-phenylpropyl isobutyrate, cinnamyl isovalerate, cinnamyl isobutyrate, ethyl cinnamate, methyl cinnamate and isopentyl cinnamate.
B: 3-phenylpropanal and 3-(*p*-cumenyl)-2-methylpropionaldehyde.
(a): Complete feed with 88% dry matter (DM), except milk replacer for veal calves (94.5% DM), and for cattle for fattening, dairy cows, dogs and cats for which the values are DM intake.
(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.

Table 5: Maximum safe concentration in feed for different target animals for (**C**) 2-phenylpropanal [05.038] (NOAEL 50 mg/kg bw per day), (**D**) α-pentylcinnamaldehyde [05.040] and α-hexylcinnamaldehyde (NOAEL 30 mg/kg bw per day), and (**E**) α-methylcinnamaldehyde [05.050] (NOAEL 220 mg/kg bw per day)

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]		
			C	D	E	C	D	E
Salmonids	2	40	1	0.6	4.4	25	15	111
Veal calves (milk replacer)	100	2,000	50	30	220	25	15	110
Cattle for fattening	400	8,000	200	120	880	22	13	97
Dairy cows	650	20,000	325	195	1430	14	9	63
Piglets	20	1,000	10	6.0	44	10	6	44
Pigs for fattening	100	3,000	50	30	220	17	10	73
Sows	200	6,000	100	60	440	17	10	73
Chickens for fattening	2	120	1	0.6	4.4	8	5	37
Laying hens	2	120	1	0.6	4.4	8	5	37
Turkeys for fattening	12	400	6	3.6	26.4	15	9	66
Dogs	15	250	7.5	4.5	33	26	16	116
Cats[c]	3	60	0.3	0.2	1.3	4	3	19

C: 2-phenylpropanal.
D: α-pentylcinnamaldehyde and α-hexylcinnamaldehyde.
E: α-methylcinnamaldehyde.
(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.

3.2.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that:

- cinnamaldehyde [05.014] is safe at the proposed maximum use level of 125 mg/kg complete feed for salmonids, veal calves and dogs. For the remaining target species, the normal use level of 25 mg/kg complete feed is considered safe;
- cinnamyl alcohol [02.017], 3-phenylpropan-1-ol [02.031], 3-(*p*-cumenyl)-2-methylpropionaldehyde [05.045], α-methylcinnamaldehyde [05.050], 3-phenylpropanal [05.080], cinnamic acid [08.022], cinnamyl acetate [09.018], cinnamyl butyrate [09.053], 3-phenylpropyl isobutyrate [09.428], cinnamyl isovalerate [09.459], cinnamyl isobutyrate [09.470], ethyl cinnamate [09.730], methyl cinnamate [09.740] and isopentyl cinnamate [09.742] are safe at the proposed maximum use level of 5 mg/kg complete feed for all target species;
- 2-phenylpropanal [05.038], α-pentylcinnamaldehyde [05.040] and α-hexylcinnamaldehyde [05.041] are safe at the proposed maximum dose 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 safe.

3.2.4. Safety for the consumer

The safety for the consumer of the compounds in CG 22, used as food flavours, has already been assessed by JECFA (WHO, 2001a) and EFSA (EFSA, 2009a,b). All these compounds are presently authorised as food flavourings without limitations.[5]

Given the use levels of CG 22 compounds to be applied in feed and the extensive metabolism and excretion in target animals (see Section 3.1.1), 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 18 compounds up to the highest proposed 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.

The available literature on cinnamaldehyde indicates that irritating and allergic reactions are commonly reported among those handling the product in the workplace (Opdyke, 1979) or otherwise exposed (Rademaker and Forsyth, 1989).

3.2.6. Safety for the environment

The additions of naturally occurring substances that will not result in a substantial increase in the concentration in the environment are exempt from further assessment. Examination of the published literature shows that this applies to five substances, namely, cinnamyl alcohol [02.017], cinnamaldehyde [05.014], cinnamic acid [08.022], cinnamyl acetate [09.018] and methyl cinnamate [09.740], which occur in the environment at levels above the application rate of 25 (for cinnamaldehyde) and 5 mg/kg feed for the remaining four compounds (data taken from the Netherlands Organisation for Applied Scientific Research (TNO) database Volatile Compounds in Food *ver.* 14.1; Burdock, 2009).[12]

The other 13 compounds, namely 3-phenylpropan-1-ol [02.031], 2-phenylpropanal [05.038], α-pentylcinnamaldehyde [05.040], α-hexylcinnamaldehyde [05.041], 3-(*p*-cumenyl)-2-methylpropional-dehyde [05.045], α-methylcinnamaldehyde [05.050], 3-phenylpropanal [05.080], cinnamyl butyrate [09.053], 3-phenylpropyl isobutyrate [09.428], cinnamyl isovalerate [09.459], cinnamyl isobutyrate [09.470], ethyl cinnamate [09.730] and isopentyl cinnamate [09.742], do not occur in the environment at levels above the application rate of 5 mg/kg feed. However, the FEEDAP Panel assumes that there is a high probability of complete hydrolysis in the target animal of the esters, cinnamyl butyrate [09.053], 3-phenylpropyl isobutyrate [09.428], cinnamyl isovalerate [09.459], cinnamyl isobutyrate [09.470], ethyl cinnamate [09.730] and isopentyl cinnamate [09.742], resulting in cinnamyl alcohol and cinnamic acid, which are naturally occurring compounds. Similarly, considering the metabolism in the target animal (see Section 3.2.1), it is expected that 3-phenylpropan-1-ol [02.031], 2-phenylpropanal [05.038], α-methylcinnamaldehyde [05.050] and 3-phenylpropanal [05.080] are metabolised to benzoic acid and that 3-(*p*-cumenyl)-2-methylpropionaldehyde [05.045] is oxidised to the corresponding carboxylic acid. Therefore, these compounds are excluded from further assessment.

For the remaining two compounds, namely α-pentylcinnamaldehyde [05.040] and α-hexylcinnamal-dehyde [05.041], the predicted environmental concentration calculation for soil (PEC_{soil}) was calculated based on the maximum proposed use level (Table 5) and compared with the trigger values for compartments set in the phase I of EFSA guidance (EFSA, 2008c).

Table 6: Predicted environmental concentration (PEC) values for α-pentylcinnamaldehyde [05.040] and α-hexylcinnamaldehyde [05.041] (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)
α-Pentylcinnamaldehyde	122-40-7	5	107	9	3
α-Hexylcinnamaldehyde	101-86-0	5	107	5	2

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

PEC_{soil} values are above the threshold of 10 μg/kg (EFSA, 2008c). The PEC for pore water, ($PEC_{pore\ water}$) is dependent on the sorption, which is different for each compound. For these calculations, the substance-dependent constants, organic carbon sorption constant (K_{oc}), molecular weight, vapour pressure and solubility, are needed. These were estimated from the Simplified Molecular Input Line Entry Specification (SMILES) notation of the chemical structure using EPIWEB 4.1 (Table 6).[13] This program was also used to derive the SMILES notation from the CAS numbers. The K_{oc} value derived from the first-order molecular connectivity index was used, as recommended by the EPIWEB program (Table 7).

[11] Technical dossier/Section II/Annex II.3.
[12] Technical dossier/Supplementary information June 2011.
[13] Available online: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm

Table 7: Physicochemical properties predicted by EPIWEB 4.1 for α-pentylcinnamaldehyde [05.040] and α-hexylcinnamaldehyde [05.041]

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)
α-Pentylcinnamaldehyde	122-40-7	6	202.30	0.06	8.55	628
α-Hexylcinnamaldehyde	101-86-0	7	216.33	0.07	2.75	1,242

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

The half-life (DT_{50} soil) was calculated using BioWin3 (Ultimate Survey Model), which gives a rating number. This rating number r was translated into a half-life using the formula 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 the BioWin3 model output.

The two substances in Table 6 have $PEC_{pore\ water}$ above 0.1 μg/L and a PEC_{soil} above 10 μg/kg. Therefore, these two substances are subjected to phase II risk assessment.

In the absence of experimental data, the phase II risk assessment was performed using ECOSAR v1.11, which estimates the half-maximal effective concentration (EC_{50}) or lethal concentration (LC_{50}) for ecotoxicologically relevant organisms from the SMILES notation of the substance. The predicted no effect concentration (PNEC) for aquatic compartment ($PNEC_{aquatic}$) was derived from the lowest toxicity value for freshwater environment by applying a UF of 1,000.

Table 8: Phase II environmental risk assessment of aquatic compartment for α-pentylcinnamaldehyde [05.040] and α-hexylcinnamaldehyde [05.041] when used as feed additives for terrestrial farm animals at the proposed maximum use level of 5 mg/kg (exposure and effect data were modelled using EPIWEB 4.1 and ECOSAR 1.11)

EU Register name Aquatic	LC_{50}[(a)] Fish (mg/L)	LC_{50} Daphnids (mg/L)	EC_{50}[(b)] Algae (mg/L)	$PNEC_{aquatic}$ (μg/L)	PEC_{sw}[(c)] (μg/L)	PEC_{sw}/ PNEC
α-Pentylcinnamaldehyde	0.2	1.5	1.8	0.2	3	15
α-Hexylcinnamaldehyde	0.2	0.6	0.9	0.2	2	10

EU: European Union.
(a): LC_{50}: The concentration of a test substance which results in a 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): PEC_{sw}: Predicted environmental concentration in surface water.

For α-pentylcinnamaldehyde and α-hexylcinnamaldehyde, the maximum and the normal proposed use levels would result PEC/PNEC ratio for surface water > 1 (Table 8). For both compounds, a use level of 0.1 mg/kg feed would not cause a risk to the fresh water environment, as shown in Table 9.

Table 9: Phase II environmental risk assessment of aquatic compartment for α-pentylcinnamaldehyde [05.040] and α-hexylcinnamaldehyde [05.041] when used as feed additives for terrestrial farm animals at the use level of 0.1 mg/kg feed (exposure and effect data were modelled using EPIWEB 4.1 and ECOSAR 1.11)

EU Register name Aquatic	$PNEC_{aquatic}$[(a)] (μg/L)	PEC_{sw}[(b)] (μg/L)	PEC_{sw}/PNEC
α-Pentylcinnamaldehyde	0.2	0.0585	0.29
α-Hexylcinnamaldehyde	0.2	0.0323	0.16

(a): $PNEC_{aquatic}$: predicted no effect concentration for aquatic compartment.
(b): PEC_{sw}: predicted environmental concentration calculation for surface water.

For both compounds, a use level of 0.1 mg/kg feed would result in a PEC_{soil} below the trigger value, thus a risk for the terrestrial compartment is not expected.

If used in fish feed at the highest proposed use level of 5 mg/kg complete feed in land-based aquaculture systems, none of the additives under assessment would result in a predicted environmental concentration of the additive (parent compound) in surface water (PEC_{swaq}) above the trigger value of 0.1 μg/L when calculated according to the guidance (EFSA, 2008c). 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, 2008c).

3.2.6.1. Conclusions on safety for the environment

For all the compounds belonging to CG 22, except α-pentylcinnamaldehyde and α-hexylcinnamaldehyde, the maximum proposed use levels (see Section 3.2.3) are considered safe for the environment. For the marine environment, the safe use level is estimated to be 0.05 mg/kg feed.

For α-pentylcinnamaldehyde and α-hexylcinnamaldehyde, a use level up to 0.1 mg/kg feed would not cause a risk for the terrestrial and fresh water compartments.

3.3. Efficacy

Since all 18 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 cinnamaldehyde [05.014] is safe at the maximum use level of 125 mg/kg complete feed for salmonids, veal calves and dogs, and at the use level of 25 mg/kg complete feed for the remaining target species; cinnamyl alcohol [02.017], 3-phenylpropan-1-ol [02.031], 3-(*p*-cumenyl)-2-methylpropionaldehyde [05.045], α-methylcinnamaldehyde [05.050], 3-phenylpropanal [05.080], cinnamic acid [08.022], cinnamyl acetate [09.018], cinnamyl butyrate [09.053], 3-phenylpropyl isobutyrate [09.428], cinnamyl isovalerate [09.459], cinnamyl isobutyrate [09.470], ethyl cinnamate [09.730], methyl cinnamate [09.740] and isopentyl cinnamate [09.742] are safe at the proposed maximum use level of 5 mg/kg complete feed for all target species; 2-phenylpropanal [05.038] α-pentylcinnamaldehyde [05.040] and α-hexylcinnamaldehyde [05.041] 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 safe.

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

Irritation and sensitisation hazards for skin and irritation for eye are recognised for the majority of the compounds under application. Respiratory exposure may also be hazardous.

For all the compounds belonging to CG 22, except α-pentylcinnamaldehyde and α-hexylcinnamaldehyde, the maximum proposed use levels are considered safe for the environment. For α-pentylcinnamaldehyde and α-hexylcinnamaldehyde, a use level up to 0.1 mg/kg feed would not cause a risk for the terrestrial and fresh water compartments.

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 22 – Aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones 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 22 – Aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones for all animal species and categories. June 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
3) Chemically defined flavourings from Flavouring Group 22 – Aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones for all animal species and categories. March 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

4) Chemically defined flavourings from Flavouring Group 22 – Aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones for all animal species and categories. July 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
5) Chemically defined flavourings from Flavouring Group 22 – Aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones for all animal species and categories. November 2016. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
6) Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Chemically Defined Flavourings – Group 22 (CDG 22 – Aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives including unsaturated ones).
7) Comments from the Member States.

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Abbreviations

ADI	acceptable daily intake
ALAT	alanine transaminase
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CDG	chemically defined group
DM	dry matter
DT_{50}	degradation half-time
EC_{50}	half-maximal effective concentration
ECOSAR	component program of EPI suite™
EEIG	European Economic Interest Grouping
EPI suite	Estimation Programs Interface (EPI) Suite™
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	The EU Flavour Information System
FL-No	FLAVIS number
GC–MS	gas chromatography–mass spectrometry
i.p.	intraperitoneal
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
NOEL	no observed effect level
NOAEL	no observed adverse effect level
PEC	predicted environmental concentration
PEC_{soil}	predicted environmental concentration for soil
$PEC_{pore\ water}$	predicted environmental concentration for pore water
$PEC_{surface\ water}$/PEC_{sw}	predicted environmental concentration for surface water
PEC_{swaq}	predicted environmental concentration of the additive (parent compound) in surface water
PNEC	predicted no effect concentration
$PNEC_{aquatic}$	predicted no effect concentration for aquatic compartment
SMILES	Simplified Molecular Input Line Entry Specification
TNO	Netherlands Organisation for Applied Scientific Research
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 aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives including unsaturated ones

The *Chemically Defined Flavourings – Group 22* (*Aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives including unsaturated ones*), in this application comprises nineteen 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% and 90% for 3-(p-Cumenyl)-2-methylpropionaldehyde.

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 *CDG22* 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 *CDG22* 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 *CDG22* 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 *CDG22* 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*.

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.