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Safety of 27 flavouring compounds providing a milky-vanilla flavour and belonging to different chemical groups for use as feed additives in all animal species (FEFANA asbl)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Noël Dierick, Giovanna Martelli, Johannes Westendorf, Montserrat Anguita, Jordi Ortuño Casanova and Paola Manini

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety of 27 compounds to provide a milky-vanilla flavour belonging to different chemical groups, when used as sensory additives in feed for all animal species. Fifteen of the 27 compounds were tested in tolerance studies in chickens for fattening, piglets and cattle for fattening. No adverse effects were observed in the tolerance studies at 10-fold the intended level. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the 15 tested compounds were safe for these species at the proposed use level and conclusions were extrapolated to all animal species. For the remaining 12 compounds, read-across from structurally similar compounds tested in tolerance trials and belonging to the same chemical group was applied. The FEEDAP Panel concluded that these 12 compounds were safe for all animal species at the proposed use level. No safety concern would arise for the consumer from the use of the 27 compounds up to the highest levels considered safe for target animals. No new data were submitted on the safety for the user that would allow the FEEDAP Panel to change its previous conclusion for 5-methylhept-2-en-4-one [07.139], 5-methylfurfural [13.001] and 4-phenylbut-3-en-2-one [07.024]. The concentrations considered safe for the target species are unlikely to have detrimental effects on the environment for all the compounds.

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Keywords: sensory additives, flavourings, tolerance studies with mixture of flavourings, milky-vanilla, safety, read-across, environment

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Correspondence: feedap@efsa.europa.eu

Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.2. Additional information.....	7
2. Data and methodologies.....	9
2.1. Data.....	9
2.2. Methodologies.....	9
3. Assessment.....	9
3.1. Conditions of use.....	10
3.2. Safety.....	10
3.2.1. Safety for the target species.....	10
3.2.1.1. Test item and feed preparation.....	10
3.2.1.2. Tolerance study in chickens for fattening.....	11
3.2.1.3. Tolerance study in weaned piglets.....	12
3.2.1.4. Tolerance study in cattle for fattening.....	12
3.2.1.5. Conclusions on the safety for the target species for the compounds tested in the tolerance studies.....	13
3.2.1.6. Extrapolation of the conclusions of the tolerance studies.....	13
3.2.1.7. Conclusions on safety for the target species.....	16
3.2.2. Safety for the consumer.....	17
3.2.3. Safety for the user.....	18
3.2.3.1. Conclusions on safety for the user.....	19
3.2.4. Safety for the environment.....	19
3.2.4.1. Conclusions on safety for the environment.....	21
4. Conclusions.....	21
5. Documentation provided to EFSA/Chronology.....	22
References.....	23
Abbreviations.....	24

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003¹ establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defined the term of the authorisation by the Commission.

The applicant, FEFANA asbl, is seeking a Community authorisation of 27 flavourings compounds (butyric acid, ethyl butyrate, ethyl isobutyrate, ethyl isovalerate, methyl isovalerate, 2-methyl-2-pentenoic acid, 6-methylhept-5-en-2-one, undecan-2-one, octan-2-one, nonan-2-one, octan-3-one, tridecan-2-one, 5-methylhept-2-en-4-one, dodecano-1,5-lactone, tetradecano-1,5-lactone, 5-methylfurfural, 4-phenylbut-3-en-2-one, p-anisyl alcohol², 4-methoxybenzaldehyde, piperonal, vanillin, p-anisyl acetate,³ benzyl benzoate, isobutyl salicylate, isopentyl salicylate, benzyl salicylate and diphenyl ether) as feed additives to be used as flavouring compounds for all animal species, except for p-anisyl alcohol and p-anisyl acetate that the request is for use in all species except fish and poultry (Table 1).

Table 1: Description of the additives

Category of additive	Sensory additive
Functional group of additives	Flavouring compounds
Description	Butyric acid, ethyl butyrate, ethyl isobutyrate, ethyl isovalerate, methyl isovalerate, 2-methyl-2-pentenoic acid, 6-methylhept-5-en-2-one, undecan-2-one, octan-2-one, nonan-2-one, octan-3-one, tridecan-2-one, 5-methylhept-2-en-4-one, dodecano-1,5-lactone, tetradecano-1,5-lactone, 5-methylfurfural, 4-phenylbut-3-en-2-one, p-anisyl alcohol, 4-methoxybenzaldehyde, piperonal, vanillin, p-anisyl acetate, benzyl benzoate, isobutyl salicylate, isopentyl salicylate, benzyl salicylate and diphenyl ether
Target animal category	All animal species except for p-anisyl alcohol and p-anisyl acetate (all species except fish and poultry)
Applicant	FEFANA asbl
Type of request	New opinion

On 11/05/2012, 24/07/2012, 26/10/2012, 05/04/2013, 25/05/2016, 20/10/2015, 26/01/2016, 10/01/2020, 28/01/2020, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ('EFSA'), in its opinions on the safety and efficacy of the products, could not conclude on the safety of butyric acid, ethyl butyrate, ethyl isobutyrate, ethyl isovalerate, methyl isovalerate, 2-methyl-2-pentenoic acid, 6-methylhept-5-en-2-one, undecan-2-one, octan-2-one, nonan-2-one, octan-3-one, tridecan-2-one, 5-methylhept-2-en-4-one, dodecano-1,5-lactone, tetradecano-1,5-lactone, 5-methylfurfural, 4-phenylbut-3-en-2-one, p-anisyl alcohol, 4-methoxybenzaldehyde, piperonal, vanillin, p-anisyl acetate, benzyl benzoate, isobutyl salicylate, isopentyl salicylate, benzyl salicylate and diphenyl ether as feed additives for all animal species due to different aspects related to safety for human health, animal health or the environment.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinions concerned. The new data have been received on 30 November 2021.

In view of the above, the Commission asks EFSA to deliver a new opinion on the above-mentioned 27 flavouring compounds as feed additives for all animal species (except for p-anisyl alcohol and p-anisyl acetate that the request is for use in all species except fish and poultry), based on the supplementary data submitted by the applicant, in accordance with Article 29(1) (a) of Regulation (EC) No 178/2002.

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 In the current assessment referred to as anisyl alcohol (synonym: 4-methoxybenzyl alcohol).

³ In the current assessment referred to as anisyl acetate (synonym: 4-methoxybenzyl acetate).

The following table lists: the aspects on which the applicant has submitted information, the species affected, the use level requested and if the tests were performed (T) or extrapolation is requested (E) (Table 2):

Table 2: Flavouring compounds (FLAVIS number and EU register name) under assessment, use levels in feed (mg/kg) proposed for the evaluation and aspects for which applicant has submitted supplementary information to be examined by EFSA and species for which the data are intended for

FLAVIS No	FAD Number Date of adoption	Name in EU register of feed additives	Requested use level (mg/kg)/ species	TT (c)	Comment Different sections for which data is being submitted
08.005	FAD-2010-0015 05/04/2013	Butyric acid	125 All animal species	T	Evaluated by EFSA at 5 mg/kg. Animal safety data, Consumer safety and ERA at 125 mg/kg.
09.039	FAD-2010-0015 05/04/2013	Ethyl butyrate	125 All animal species	E	Evaluated by EFSA at 5 mg/kg. Animal safety data, Consumer safety and ERA at 125 mg/kg.
09.413	FAD-2010-0015 05/04/2013	Ethyl isobutyrate	25 All animal species	E	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety data, Consumer safety and ERA at 25 mg/kg.
09.447	FAD-2010-0015 05/04/2013	Ethyl isovalerate	25 All animal species	T	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety data, Consumer safety and ERA at 25 mg/kg.
09.462	FAD-2010-0015 05/04/2013	Methyl isovalerate	5 All animal species	E	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.
08.055	FAD-2010-0124 25/05/2016	2-Methyl-2-pentenoic acid	5 All animal species	T	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.
07.015	FAD-2010-0074 20/10/2015	6-Methylhept-5-en-2-one	4.5 All animal species	T	Evaluated by EFSA at 0.3–0.5 mg/kg. Animal safety data, Consumer safety and ERA at 4.5 mg/kg.
07.016	FAD-2010-0074 20/10/2015	Undecan-2-one	10 All animal species	E	Evaluated by EFSA at 0.3–0.5 mg/kg. Animal safety data, Consumer safety and ERA at 10 mg/kg.
07.019	FAD-2010-0074 20/10/2015	Octan-2-one	10 All animal species	E	Evaluated by EFSA at 0.3–0.5 mg/kg. Animal safety data, Consumer safety and ERA at 10 mg/kg.
07.020	FAD-2010-0074 20/10/2015	Nonan-2-one	10 All animal species	T	Evaluated by EFSA at 0.3–0.5 mg/kg. Animal safety data, Consumer safety and ERA at 10 mg/kg.
07.062	FAD-2010-0074 20/10/2015	Octan-3-one	10 All animal species	E	Evaluated by EFSA at 0.3–0.5 mg/kg. Animal safety data, Consumer safety and ERA at 10 mg/kg.
07.103	FAD-2010-0074 20/10/2015	Tridecan-2-one	10 All animal species	E	Evaluated by EFSA at 0.3–0.5 mg/kg. Animal safety data, Consumer safety and ERA at 10 mg/kg.

FLAVIS No	FAD Number Date of adoption	Name in EU register of feed additives	Requested use level (mg/kg)/ species	TT (c)	Comment Different sections for which data is being submitted
07.139 (d)	FAD-2010-0412 10/01/2020	5-Methylhept-2-en-4-one	5 All animal species	T	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg. User safety data.
10.008	FAD-2010-0097 26/10/2012	Dodecano-1,5-lactone	25 All animal species	T	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety data, Consumer safety and ERA at 25 mg/kg.
10.016	FAD-2010-0097 26/10/2012	Tetradecano-1,5-lactone	5 All animal species	E	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.
13.001	FAD-2010-0118 26/01/2016	5-Methylfurfural	5 All animal species	T	Evaluated by EFSA at 0.3–0.5 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg. User safety data.
07.024 (d)	FAD-2010-0417 28/01/2020	4-Phenylbut-3-en-2-one	All animal species	T	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety & toxicity data, Consumer safety and ERA at 5 mg/kg. User safety data.
02.128	FAD-2010-0028 24/07/2012	p-Anisyl alcohol	5 All animal species except fish and poultry	E	Withdrawn in fish & poultry (as communicated to the EC by letter on 2016-07-04) Evaluated by EFSA at 1 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.
05.015	FAD-2010-0028 24/07/2012	4-Methoxybenzaldehyde	25 All animal species	T	Evaluated by EFSA at 1 mg/kg. Animal safety data, Consumer safety and ERA at 25 mg/kg.
05.016	FAD-2010-0028 24/07/2012	Piperonal	5 All animal species	T	Evaluated by EFSA at 0.3–0.5 mg/kg. Animal safety and ERA at 5 mg/kg. Consumer safety for all species.
05.018	FAD-2010-0028 24/07/2012	Vanillin	125 All animal species	T	Evaluated by EFSA at 25 mg/kg. Animal safety and ERA at 125 mg/kg. Consumer safety for poultry & fish.
09.019	FAD-2010-0028 24/07/2012	p-Anisyl acetate	5 All animal species except fish and poultry	E	Withdrawn in fish & poultry (as communicated to the EC by letter on 2016-07-04) Evaluated by EFSA at 1 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.
09.727	FAD-2010-0028 24/07/2012	Benzyl benzoate	5 All animal species	T	Evaluated by EFSA at 1–1.5 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.
09.750	FAD-2010-0028 24/07/2012	Isobutyl salicylate	5 All animal species	E	Evaluated by EFSA at 1 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.

FLAVIS No	FAD Number Date of adoption	Name in EU register of feed additives	Requested use level (mg/kg)/ species	TT (c)	Comment Different sections for which data is being submitted
09.751	FAD-2010-0028 24/07/2012	Isopentyl salicylate	5 All animal species	E	Evaluated by EFSA at 1 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.
09.752	FAD-2010-0028 24/07/2012	Benzyl salicylate	25 All animal species	T	Evaluated by EFSA at 1 mg/kg. Animal safety data, Consumer safety and ERA at 25 mg/kg.
04.035	FAD-2010-0054 11/05/2012	Diphenyl ether	5 All animal species	T	Evaluated by EFSA at 1 mg/kg. Animal safety data, Consumer safety and ERA at 5 mg/kg.

1.2. Additional information

The list of the 27 flavouring compounds currently authorised for food⁴ and feed⁵ uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000⁶ and the corresponding EFSA opinion is given in Table 3.

Table 3: Flavouring compounds under assessment, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/20002, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding FEEDAP opinion

CG	Chemical group	Product (EU register name)	FLAVIS No	Year
01	Straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes	Butyric acid	08.005	2013
		Ethyl butyrate	09.039	
		Ethyl isobutyrate	09.413	
		Ethyl isovalerate	09.447	
		Methyl isovalerate	09.462	
03	a, β-Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters	2-Methyl-2-pentenoic acid	08.055	2016a
05	Saturated and unsaturated aliphatic secondary alcohol/ketones/esters with esters containing secondary alcohols	6-Methylhept-5-en-2-one	07.015	2015
		Undecan-2-one	07.016	
		Octan-2-one	07.019	
		Nonan-2-one	07.020	
		Octan-3-one	07.062	
		Tridecan-2-one	07.103	
		5-Methylhept-2-en-4-one	07.139	2020a
09	Primary aliphatic saturated or unsaturated alcohols/aldehydes/acids/acetals/esters with a second primary, secondary or tertiary oxygenated functional group	Dodecano-1,5-lactone	10.008	2012a
		Tetradecano-1,5-lactone	10.016	
14	Furfuryl and furane derivatives	5-Methylfurfural	13.001	2016b
21	Aromatic ketones, secondary alcohols and related esters	4-Phenylbut-3-en-2-one	07.024	2020b

⁴ 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.

⁵ European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf

⁶ 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.

CG	Chemical group	Product (EU register name)	FLAVIS No	Year
23	Benzyl alcohols, aldehydes, acids, esters and acetals	Anisyl alcohol (4-methoxybenzyl alcohol)	02.128	2012b
		4-Methoxybenzaldehyde (anisaldehyde)	05.015	
		Piperonal	05.016	
		Vanillin	05.018	
		Anisyl acetate (4-methoxybenzyl acetate)	09.019	
		Benzyl benzoate	09.727	
		Isobutyl salicylate	09.750	
		Isopentyl salicylate	09.751	
		Benzyl salicylate	09.752	
26	Aromatic ethers including anisole derivatives	Diphenyl ether	04.035	2012c

In the context of the re-evaluation of feed flavourings, the FEEDAP Panel issued 39 opinions dealing with 568 compounds. For about 35% of the compounds assessed, in the absence of data (tolerance studies and/or toxicological studies with the additives under assessment from which a no observed adverse effect level (NOAEL) could be derived) or because of the unsuitability of the available toxicological data, the FEEDAP Panel could not conclude on the safety for target animals of the compounds at the maximum use level proposed by the applicant. The FEEDAP Panel, however, was in each case able to identify a lower safe use level for all animal species, based on the available toxicological information or, more commonly, based on the application of the threshold of toxicological concern (TTC) approach. The FEEDAP Panel also concluded that no safety concern would arise for the consumer or for the environment from the use of these compounds at the identified safe levels in feed.

For a number of substances, the safe use level identified by the FEEDAP Panel was lower than that typically used in feed and, in some cases, considered by the industry to be too low to allow an effective use as flavouring. The European Commission gave the applicant the possibility to submit complementary information with the aim to demonstrate the safety of the proposed use levels and allow a revision of those EFSA opinions which the industry found restrictive. The applicant recognised that to provide tolerance or toxicological studies for each individual, flavouring would not be feasible and would have required a very high number of animals. As an alternative, the applicant proposed the use of tolerance studies designed to test a number of flavouring compounds simultaneously in a mixture, using concentrations which reflected their commercial application and an overdose. The intention was then to conclude on a safe level in feed for each component of the mixture based on their concentration in the mixture and the outcome of the tolerance study.

Four different mixtures (characterised by different olfactory notes, i.e. milky-vanilla, toasted cereal, herbal and TuttiFrutti) with a total of 68 compounds have been designed to be tested in three major species, chickens for fattening, piglets and cattle for fattening, for a total of 12 tolerance trials. Based on the structural similarity within a chemical group, the applicant also proposed the extrapolation of the conclusions from some of the compounds tested in the tolerance trials to structurally similar compounds belonging to the same chemical group, giving an overall total of 133 compounds. Data on residues in manure samples (excreta from chickens and in faeces and urine from piglets and cattle for fattening) from animals fed the mixture of additives at the maximum recommended use level were also collected to be used in the assessment of the safety for the environment.

As the tolerance studies were started in October 2016, over a 3-year planning, they were designed to follow the provisions present in the guidance on sensory additives (EFSA FEEDAP Panel, 2012d), which was in place at that time. The FEEDAP Panel exceptionally accepts the approach.

This application deals with the results of tolerance studies made with one of the four mixtures tested and the implications for target animal safety, consumer safety and the environment.

This application covers 27 compounds under assessment, belonging to several chemical groups (CGs), namely CG 1, 3, 5, 9, 14, 21, 23 and 26, when used as feed flavourings for all animal species

which were assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2012a,b,c, 2013, 2015, 2016a,b, 2020a,b).

For a number of compounds (about 9%, 49 compounds) in the absence of specific studies to assess the safety for the user, the FEEDAP Panel cannot conclude on the safety for the users when handling the additives.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information to previous applications on the same products.⁷

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the chemically defined groups in animal feed are valid and applicable for the current application.⁸

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of 27 flavourings belonging to different chemically defined groups is in line with the principles laid down in Regulation (EC) No 429/2008⁹ and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012d), guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e) and guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The additives under assessment are 27 compounds belonging to several chemical groups, namely CG 1, 3, 5, 9, 14, 21, 23 and 26, intended for use as sensory additives (functional group: flavouring compounds) in feed for all animal species.¹⁰

In previous opinions of the FEEDAP Panel (EFSA FEEDAP Panel, 2012a,b,c, 2013, 2015, 2016a,b, 2020a,b), the 27 additives under assessment were fully characterised and evaluated for their safety and efficacy as flavouring substances. For all compounds, the FEEDAP Panel could not conclude on the safety for target animals at the maximum use level proposed by the applicant. The Panel, however, was in each case able to identify a safe use level for all animal species, lower than the maximum proposed use level, based on the available toxicological information or, more commonly, based on the application of the TTC approach. The Panel also concluded that no safety concern would arise for the consumer or the environment from the use of these compounds at the identified safe levels in feed but did not conclude at the maximum use level proposed by the applicant.

The applicant has provided new data to address the limitations previously identified regarding the safety for the target species and the safety for the environment. The new data submitted consist of tolerance studies in chickens for fattening, piglets and cattle for fattening, performed with a mixture of the 16 flavourings under assessment. One compound, 2-acetylfuran [13.054] was tested in the mixture but not assessed in the current opinion. Data on residues in manure samples (excreta from chickens and in faeces and urine from piglets and cattle for fattening) from animals fed the mixture of additives at the maximum recommended use level were also collected to allow the FEEDAP Panel to review its assessment of the safety for the environment. For the remaining 12 compounds under assessment,

⁷ FEED dossiers' reference: FAD-2010-0015, FAD-2010-0028, FAD-2010-0054, FAD-2010-0074, FAD-2010-0097, FAD-2010-0118, FAD-2010-0124, FAD-2010-0412, FAD-2010-0417.

⁸ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0015.pdf>; <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0028.pdf>; <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0054.pdf>; <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0074.pdf>; <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0075.pdf>; <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0124.pdf>; <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0125.pdf>.

⁹ 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.

¹⁰ All animal species, except in case of anisyl alcohol and anisyl acetate for which the opinion is requested for all animal species except fish and poultry.

which were not tested in the tolerance trials, the applicant proposed to extrapolate the conclusions for structurally similar compounds tested in the tolerance studies.

For three compounds (5-methylhept-2-en-4-one [07.139], 5-methylfurfural [13.001] 4-phenylbut-3-en-2-one [07.024] belonging to CGs 5, 14 and 21, respectively), no studies to assess the safety for the user were submitted for previous assessments. Therefore, the FEEDAP Panel could not conclude on the safety for the users when handling the additive at that time (EFSA FEEDAP Panel, 2016b, 2020a, b). No new data were submitted on the safety for the user.

3.1. Conditions of use

The maximum recommended levels proposed by the applicant for each compound tested in the mixture of flavourings is shown in Table 4 (referring to onefold level). The conditions of use for the remaining 12 compounds are summarised in Table 5 (Section 3.2.1.6).

3.2. Safety

3.2.1. Safety for the target species

3.2.1.1. Test item and feed preparation

The mixture tested in tolerance studies is named 'milky-vanilla' and includes 16 flavouring compounds belonging to several chemical groups. The FEEDAP Panel notes that 2-acetylfuran was included in the mixture, but the assessment of this substance is not in the scope of the current work. Therefore, herein the Panel may refer to a mixture of 15 compounds. The individual components of the mixture, their FLAVIS numbers, the maximum recommended dose (MRD, 1×) proposed by the applicant and the two overdoses tested, 3× MRD or 10× MRD per kg complete feed, are described in Table 4.

Table 4: Individual components of the mixture and intended dosages tested in tolerance trials

CG	EU register name	FLAVIS No	1× MRD	3× MRD	10× MRD
			mg/kg complete feed		
01	Butyric acid	08.005	125	375	1,250
01	Ethyl isovalerate	09.447	25	75	250
03	2-Methyl-2-pentenoic acid	08.055	5	15	50
05	6-Methylhept-5-en-2-one	07.015	4.5	13.5	45
05	Nonan-2-one	07.020	10	30	100
05	5-Methylhept-2-en-4-one	07.139	5	15	50
09	Dodecano-1,5-lactone	10.008	25	75	250
14	5-Methylfurfural	13.001	5	15	50
14	2-Acetylfuran	13.054	0.5	1.5	5
21	4-Phenylbut-3-en-2-one	07.024	5	15	50
23	4-Methoxybenzaldehyde (anisaldehyde)	05.015	25	75	250
23	Piperonal	05.016	5	15	50
23	Vanillin	05.018	125	375	1,250
23	Benzyl benzoate	09.727	5	15	50
23	Benzyl salicylate	09.752	25	75	250
26	Diphenyl ether	04.035	5	15	50

EU: European Union; FLAVIS No: EU Flavour Information System numbers; MRD: maximum recommended dose.

¹¹ Sampling time: day 1, 7, 16, 20 and 27 (chickens for fattening); day 1, 7, 14, 21, 28 and 35 (piglets); day 12, 19, 33, 35, 42 and 48 (cattle for fattening).

Homogeneity of the test product was tested on 10× MRD samples at different time intervals,¹⁴ taking 10 individual subsamples by monitoring 4-methoxybenzaldehyde (anisaldehyde), a compound with one of the highest recoveries, as a marker. The coefficient of variation ranged between 4.1% and 4.7% in poultry feed, between 2.6 and 9.0% in feed for piglets and between 5.4% and 8.6% in feed for cattle for fattening.

3.2.1.2. Tolerance study in chickens for fattening

A total of 800 1-day-old male chickens for fattening (Ross 308) were distributed to 32 pens in groups of 25 animals and allocated to four dietary treatments (eight replicates per treatment), blocking applied depending on the situation of the pen in the room. Two basal diets (starter up to day 14, and grower from day 15 to 36) based on maize and soya bean meal were either not supplemented (control) or supplemented with the mixture to provide 1× MRD, 3× MRD or 10× MRD per kg feed (confirmed by analysis). The test mixture was added daily to the basal diet. Feed from the previous day was removed from the feeder in each pen and weighed. Animals were under study for 36 days, diets were offered in mash form and presented coccidiostats for the whole duration of the study.

Mortality and health status were checked daily, and dead animals were necropsied. Animals were weighed on days 1, 14 and 35 (pen basis); feed intake was registered per pen and feed to gain ratio was calculated. Blood samples were taken from two birds per pen (one on day 35 and the other one on day 36) for haematology¹⁵ and blood biochemistry¹⁶ (the birds were randomly selected at the beginning of the study). At 36 days of age, two chickens from each pen from control and 10× MRD treatment groups were sacrificed and used for necropsy and gross pathology evaluations. The basic study design was a randomised complete block design of four dietary treatments allocated in eight blocks, with pen location as block criteria. An analysis of variance (ANOVA) was done with the data (pen basis, individual for the blood parameters) and considering the treatment and the block as the main effects. Group means were compared with Tukey test. The significance level was set at 0.05.

The birds were in general good health throughout the study (mortality range: 1.1–2.7%, not statistically different between treatments). The feed intake and final body weight of the animals were lower (16% and 19%, respectively) than the ones expected for the genotype of birds used, but this could be due partly to the use of mash feed and the low body weight at the first day of age.

Birds receiving the mixture at 10× MRD showed significantly lower ($p < 0.05$) body weight at 35 days, ADG and ADFI (BW 1,748 g; ADG 48.8 g and ADFI 78.5 g) when compared to both control animals (BW 1,851 g; ADG 51.8 g and ADFI 82.4 g) and 3× MRD (BW 1,848 g; ADG 51.7 g and ADFI 82.0 g). No significant differences were observed between chickens receiving 1× MRD and 10× MRD. No differences were observed in the feed to gain ratio among the four groups. These results indicate that animals receiving the highest dosage of the product ingested less food, likely due to excessive flavour.

Dietary treatment had no significant effect on the haematological profile of chickens for fattening at the end of the study, except for mean corpuscular haemoglobin (MCH) values which were slightly lower, although significant ($p < 0.05$), in chickens receiving the mixture at 10× MRD relative to the control diet (50.8 vs. 52.5 pg). No significant effects of dietary treatment on any of the serum biochemical parameters were observed, except for a significantly higher creatinine in chickens of group receiving 3× MRD (0.211 mg/dL) when compared with both the control diet (0.186 mg/dL) and group 10× MRD (0.189 mg/dL). This effect was not treatment-related and considered to be of marginal biological significance.

¹² Recovery at 1× MRD: 59–112% for poultry, 63–112% for piglets and 58–120% for cattle for fattening.

¹³ Recovery at 3× MRD: 68–109% for poultry, 63–106% for piglets and 68–117% for cattle for fattening.

¹⁴ Sampling time: day 1, 14 and 27 (chickens for fattening); day 1, 14 and 35 (piglets); day 1, 26 and 48 (cattle for fattening).

¹⁵ Total count for erythrocytes, packed cell volume, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total and differential counts for leucocytes, platelet counts.

¹⁶ Sodium, potassium, chloride, calcium, phosphate, magnesium, total protein, albumin, globulin, glucose, uric acid, cholesterol, creatinine, bilirubin, acute phase protein, amylase, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and creatine kinase.

Concerning gross pathology, liver weight, expressed as a percentage of body weight, was higher in chickens receiving 10× MRD of the test product compared with animals on the control diet (2.67% vs. 2.39%). No other differences were observed in the remaining organs.

The FEEDAP Panel concludes that the components of the mixture are safe under the proposed conditions of use with a margin of safety of 10.

3.2.1.3. Tolerance study in weaned piglets

A total of 144 Piétrain × (Landrace × Large White) weaned piglets of 33 days of age, half females and half males, with an initial body weight of 8.3 kg, were distributed according to body weight and sex to 36 pens each containing four animals (two males and two females), representing nine replicates per treatment. Two basal diets (pre-starter, up to day 14 of trial and starter, from 15 to 42 day of trial), mainly based on maize and soya bean meal, were either not supplemented (control) or supplemented with the mixture to provide: 1× MRD, 3× MRD or 10× MRD per kg feed (confirmed by analysis). Feed was offered on ad libitum basis in mash form for 42 days.

Mortality and health status were checked daily. Piglets were individually weighed on days 1, 14 and 42 of trial. Feed intake was registered per pen and average daily gain, average daily feed intake and feed to gain ratio were calculated. At the end of the experiment (day 42 of trial), blood samples were taken from two piglets per pen (one male and one female randomly selected at the beginning of the trial) for haematology¹⁵ and blood biochemistry.¹⁷ At 42 days of age, one piglet from each pen from the control group and the group receiving the mixture at 10× MRD was sacrificed and used for gross pathology evaluations. The experimental unit was the pen for production traits and the individual animal for blood parameters. All data were analysed by using a generalised linear model. The treatment and the block were the main effects for production traits; the treatment, the block and the sex were the main effects for blood parameters. Tukey's test was used as post hoc analysis. The significance level was set at $p < 0.05$.

The health status of the piglets was good throughout the study. Two animals died in the onefold group (due to enteritis and pneumonia). No differences were observed among groups for body weight (mean value for final BW 35.3 kg) and daily feed intake (mean value 1,067 g) while feed to gain ratio was significantly lower in each of the treatment groups (1.63, 1.64 and 1.63 for 1×, 3× and 10× MRD) compared to the control group (1.72).

As concerns blood analyses, no significant differences were observed for haematology and biochemical analyses of plasma. With respect to blood serum, glucose concentration was significantly higher in pigs receiving 1× MRD (119 mg/dL) when compared to animals receiving 10× MRD (113 mg/dL); creatinine concentration was significantly higher in the 10× MRD (1.11 mg/dL) than in the 3× MRD treatment group (1.02 mg/dL); total protein and albumin concentrations were significantly higher in the 1× MRD (54.3 g/L and 32.3 g/L, respectively) than in the 3× MRD treatment group (52.1 g/L and 30.0 g/L, respectively) and phosphorous concentration was significantly higher in pigs receiving 1× MRD (10.0 mg/dL) compared to control animals (9.4 mg/dL). These effects were not dose-related and considered of low biological relevance.

At necropsy, no significant macroscopic lesions were observed.

The FEEDAP Panel concludes that the components of the mixture are safe under the proposed conditions of use with a margin of safety of 10.

3.2.1.4. Tolerance study in cattle for fattening

A total of 24 bulls (Holstein, 345 kg body weight) were used for the study. The bulls were housed in individual pens (2.90 × 1.97 m; 3 m² net space; natural lighting) and the four dietary treatments were allocated considering the body weight of the animals (six replicates per treatment) in a random complete block design. Before the start of the experimental phase, the bulls received a common mash concentrate for 14–28 days to collect basal data (blood samples, body weight and feed intake). From the start of the study, the animals were fed the test concentrate and straw. The test concentrate was based on maize grain meal, barley grain meal, maize gluten feed and wheat middlings and was either not supplemented (control) or supplemented with the mixture to provide 1× MRD, 3× MRD or 10× MRD per kg concentrate feed (confirmed by analysis). Feed was prepared daily, and the animals had

¹⁷ Sodium, potassium, chloride, calcium, phosphate, magnesium, total protein, albumin, globulin, glucose, uric acid, cholesterol, creatinine, bilirubin, acute phase protein, amylase, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), creatine kinase, prothrombin time and fibrinogen.

free access to the mash concentrate and to straw in two separate feeders. Feed from the previous day was removed from the feeder in each pen and weighed. Water was offered ad libitum in each pen. Although the duration of the study was planned to be 42 days, finally it was extended to 49 days. Mortality and health status were checked every day. Animals were weighed on days 1, 7, 21, 42 and 49, while feed intake was registered daily for concentrate and straw; feed to gain ratio was calculated. Blood samples were taken on days 1, 7 and 49 from all animals for haematology¹⁵ and blood biochemistry.¹⁸ An ANOVA was carried out with the pen as the experimental unit. The significance level was set at 0.05.

The general health of the animals was good throughout the study and no animals died. For the overall period, there were no statistically significant differences in final body weight (control group 427 kg), average daily weight gain (control group 1.68 kg/day), feed intake (concentrate and straw 9.8 kg) or feed to gain ratio (control group 5.90) among treatments. Regarding the blood haematology and biochemistry data, no differences were observed between treatments.

The study showed no negative effects when the additive was added up to 10-fold of the MRD in the concentrate. Considering the intake of straw, the levels tested would correspond to 0.87, 2.58 and 8.55× the MDR. As the intake of concentrate was about 85% of the total dry matter intake of the animals, the real exposure to the additive was lower than the one intended in the conditions of use.

Consequently, the FEEDAP Panel concludes that the components of the mixture are safe under the proposed conditions of use with a margin of safety of at least at 8.5.

3.2.1.5. Conclusions on the safety for the target species for the compounds tested in the tolerance studies

Based on the tolerance studies in chickens for fattening, piglets and cattle for fattening in which no adverse effects were seen at intended 10-fold overdose, the FEEDAP Panel considers that the 15 compounds are safe for these species at the proposed use level.

As the margin of safety is similar in all species, the conclusions are extrapolated to all animal species for all the 15 compounds tested.

3.2.1.6. Extrapolation of the conclusions of the tolerance studies

For the remaining 12 compounds not tested in the tolerance trials, namely ethyl butyrate [09.039], ethyl isobutyrate [09.413], methyl isovalerate [09.462], undecan-2-one [07.016], octan-2-one [07.019], octan-3-one [07.062], tridecan-2-one [07.103], tetradecano-1,5-lactone [10.016], anisyl alcohol [02.128], anisyl acetate [09.019], isobutyl salicylate [09.750] and isopentyl salicylate [09.751], the applicant proposed to extrapolate the conclusions for structurally similar compounds tested in the tolerance studies and belonging to the same chemical group.

The proposed conditions of use for the 12 compounds candidate for read-across are summarised in Table 5.

Table 5: Conditions of use for the 12 compounds not tested in the tolerance trials

Chemical Group	Product (EU register name)	FLAVIS No	All animal species (mg/kg)
01	Ethyl butyrate	09.039	125
	Ethyl isobutyrate	09.413	25
	Methyl isovalerate	09.447	25
05	Undecan-2-one	07.016	10
	Octan-2-one	07.019	10
	Octan-3-one	07.062	10
	Tridecan-2-one	07.103	10
09	Tetradecano-1,5-lactone	10.016	5

¹⁸ Alkaline phosphatase, amylase, gamma-glutamyl transferase, alanin aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, calcium, phosphate, magnesium, potassium, sodium, chloride, cholesterol, lactic acid, albumin, total protein, urea, creatinine, glucose, biliary salts.

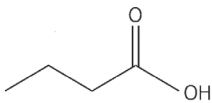
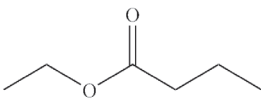
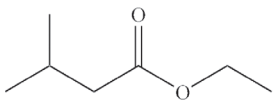
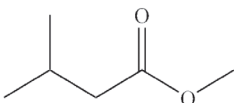
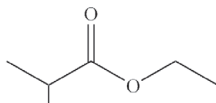
Chemical Group	Product (EU register name)	FLAVIS No	All animal species (mg/kg)
23	Anisyl alcohol	07.224	5
	Anisyl acetate	09.019	5
	Isobutyl salicylate	09.750	5
	Isopentyl salicylate	09.751	5

Read-across has been widely applied in the risk assessment of food and feed flavourings. Based on considerations related to structural and metabolic similarities, flavourings are grouped into chemical groups as defined in Annex I of Regulation (EC) No 1565/2000 and structural groups named Flavouring Group Evaluation (FGE). According to the guidance on the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012), 'The conclusions obtained for an individual flavouring may be extended to other flavourings belonging to the same structural group (e.g. an FGE).'

The application of read-across within a chemical group is applied on a case-by-case basis, considering the structural features, the physico-chemical properties and the expected reactivity of the compounds under assessment, as discussed in the paragraphs below.

Chemical group 1

The chemical structures of the compounds under assessment belonging to CG 1 are shown in Figure 1. The applicant proposed to read-across from butyric acid [08.005] to ethyl butyrate [09.039], and from ethyl isovalerate [09.447] to ethyl isobutyrate [09.413] and methyl isovalerate [09.462]. The FEEDAP Panel considers that the proposal for read-across is justified by the structural and metabolic similarity within the two groups of compounds, as shown in Figure 1. Target animals have esterases, which split the esters into the corresponding fatty acids (butyric acid and valerianic acid) and alcohols (methyl- and ethyl alcohol), which are finally converted into CO₂ (reviewed in EFSA FEEDAP Panel, 2013).

CG 1		
Butyric acid [08.005] 	Ethyl butyrate [09.039]* 	
Ethyl isovalerate [09.447] 	Methyl isovalerate [09.462]** 	Ethyl isobutyrate [09.413]** 

* proposed extrapolation from [08.005]; ** proposed extrapolation from [09.447]

Figure 1: Chemical structures and FLAVIS number of the compounds belonging to chemical group 1 for which read-across is proposed

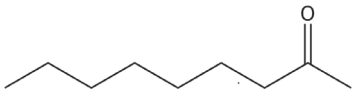
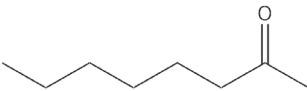
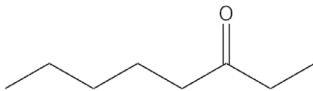
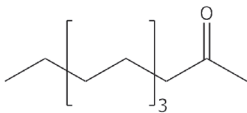
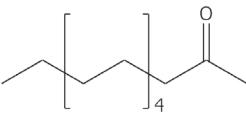
Considering that no adverse effects were observed for butyric acid [08.005] when tested in the tolerance studies in chickens, piglets and cattle for fattening up to 1,250 mg/kg feed and considering the structural similarity of the compound tested with ethyl butyrate [09.039], the FEEDAP Panel concludes that the use of ethyl butyrate [09.039] at 125 mg/kg complete feed is safe for all animal species.

Similarly, considering that no adverse effects were observed for ethyl isovalerate [09.447] when tested up to 250 mg/kg in the tolerance studies in chickens, piglets and cattle for fattening, and considering the structural similarity of the compound tested with the two candidate compounds for read-across, the FEEDAP Panel concludes that the use of ethyl isobutyrate [09.413] at 25 mg/kg

complete feed and the use of methyl isovalerate [09.462] at 5 mg/kg complete feed is safe for all animal species.

Chemical group 5

The chemical structures of the compounds under assessment belonging to CG 5 are shown in Figure 2. The applicant proposed to read-across from nonan-2-one [07.020] to undecan-2-one [07.016], octan-2-one [07.019], octan-3-one [07.062] and tridecan-2-one [07.103]. The FEEDAP Panel considers that the proposal for read-across is justified by the structural and metabolic similarity among the five compounds, as shown in Figure 2. The ketones will be reduced via ketoreductases and either conjugated with glucuronide or further fragmented to acetyl-CoA. Target animals have the ability to metabolise ketones via the activity of reductases and conjugation reactions (reviewed in EFSA FEEDAP Panel, 2015).

CG 3		
Nonan-2-one [07.020] 	Octan-2-one [07.019]* 	Octan-3-one [07.062]* 
Undecan-2-one [07.016]* 	Tridecan-2-one [07.103]* 	

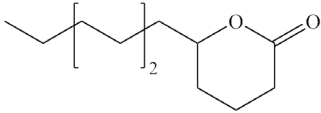
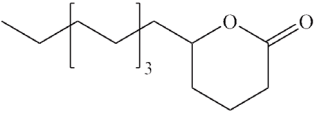
* proposed extrapolation from [07.020]

Figure 2: Chemical structures and FLAVIS number of the compounds belonging to chemical group 5 for which read-across is proposed

Considering that no adverse effects were observed for nonan-2-one [07.020] when tested up to 100 mg/kg in the tolerance studies in chickens, piglets and cattle for fattening, and considering the structural similarity between the four compounds, the FEEDAP Panel concludes that the use of undecan-2-one [07.016], octan-2-one [07.019], octan-3-one [07.062] and tridecan-2-one [07.103] at 10 mg/kg complete feed is safe for all animal species.

Chemical group 9

The chemical structures of the compounds under assessment belonging to CG 9 are shown in Figure 3. The applicant proposed to read-across from dodecano-1,5-lactone [10.008] to tetradecano-1,5-lactone [10.016]. The FEEDAP Panel considers that the proposal for read-across is justified by the structural and metabolic similarity between the two compounds, as shown in Figure 3. The two compounds are delta lactones, which are hydrolysed to 5-hydroxycarboxylic acids, undergo β -oxidation and cleavage and are finally converted to CO₂ (reviewed in EFSA FEEDAP Panel, 2012a).

CG 9	
Dodecano-1,5-lactone [10.008] 	Tetradecano-1,5-lactone [10.016]* 

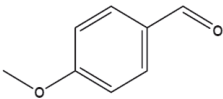
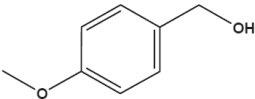
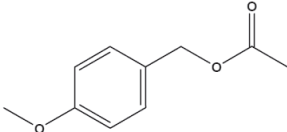
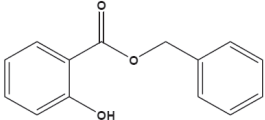
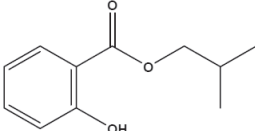
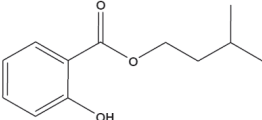
*proposed extrapolation from [10.008]

Figure 3: Chemical structures and FLAVIS number of the compounds belonging to chemical group 9 for which read-across is proposed

Considering that no adverse effects were observed for dodecano-1,5-lactone [10.008] when tested up to 250 mg/kg in the tolerance studies in chickens, piglets and cattle for fattening, and considering the structural similarity between the two compounds, the FEEDAP Panel concludes that the use of tetradecano-1,5-lactone [10.016] at 5 mg/kg complete feed is safe for all animal species.

Chemical Group 23

The chemical structures of the compounds under assessment belonging to CG 23 are shown in Figure 4. The applicant proposed to read-across from 4-methoxybenzaldehyde (anisaldehyde) [05.015] to anisyl alcohol [02.128] and anisyl acetate [09.019], and from benzyl salicylate [09.752] to isobutyl salicylate [09.750] and isopentyl salicylate [09.751]. The FEEDAP Panel considers that the proposal for read-across is justified by the structural and similarity and the common metabolic pathways within the two groups of compounds, as shown in Figure 4. Anisyl acetate is hydrolysed to anisyl alcohol, which is then oxidised to anisaldehyde. Salicylic acid esters are hydrolysed in the gastro-intestinal tract and the liver, yielding the corresponding alcohols (benzyl alcohol and aliphatic alcohols) and salicylic acid. Salicylic acid, the common moiety, is further metabolised in the liver via conjugation with glycine (or ornithine in poultry) and subsequent formation of salicyluric acid, but also glucuronide conjugation (reviewed in EFSA FEEDAP Panel, 2012b).

CG 23		
4-Methoxybenzaldehyde [05.015] 	Anisyl alcohol [02.128]* 	Anisyl acetate [09.019]* 
Benzyl salicylate [09.752] 	Isobutyl salicylate [09.750]** 	Isopentyl salicylate [09.751]** 

* proposed extrapolation from [05.015], ** proposed extrapolation from [09.752]

Figure 4: Chemical structures and FLAVIS number of the compounds belonging to chemical group 23 for which read-across is proposed

Considering that no adverse effects were observed for 4-methoxybenzaldehyde (anisaldehyde) [05.015] and benzyl salicylate [09.752] when tested up to 250 mg/kg in the tolerance studies in chickens, piglets and cattle for fattening, and considering the structural similarity between the two groups of compounds, the FEEDAP Panel concludes that the use of anisyl alcohol [02.128], anisyl acetate [09.019], isobutyl salicylate [09.750] and isopentyl salicylate [09.751] at 5 mg/kg complete feed is safe for all animal species, with the exception of fish and poultry for anisyl alcohol [02.128] and anisyl acetate [09.019].

3.2.1.7. Conclusions on safety for the target species

Based on the results of the tolerance studies in chickens for fattening, piglets and cattle for fattening, and read-across from the compounds tested to structurally similar compounds belonging to the same chemical group, the FEEDAP Panel concludes that the 27 compounds are safe for the target species at the proposed use level.

The conclusions of the FEEDAP Panel on the maximum safe concentration of the 27 compounds in complete feed for all animal species are summarised in Table 6.

Table 6: Maximum safe concentration in feed (mg/kg) for all animal species for the 27 compounds belonging to different chemical groups

CG	Product (EU register name)	FLAVIS No	All animal species (mg/kg complete feed)
01	Butyric acid	08.005	125
	Ethyl butyrate	09.039	125
	Ethyl isobutyrate	09.413	25
	Ethyl isovalerate	09.447	25
	Methyl isovalerate	09.462	5
03	2-Methyl-2-pentenoic acid	08.055	5
05	6-Methylhept-5-en-2-one	07.015	4.5
	Undecan-2-one	07.016	10
	Octan-2-one	07.019	10
	Nonan-2-one	07.020	10
	Octan-3-one	07.062	10
	Tridecan-2-one	07.103	10
	5-Methylhept-2-en-4-one	07.139	5
09	Dodecano-1,5-lactone	10.008	25
	Tetradecano-1,5-lactone	10.016	5
14	5-Methylfurfural	13.001	5
21	4-Phenylbut-3-en-2-one	07.024	5
23	Anisyl alcohol*	02.128	5
	4-Methoxybenzaldehyde (anisaldehyde)	05.015	25
	Piperonal	05.016	5
	Vanillin	05.018	125
	Anisyl acetate*	09.019	5
	Benzyl benzoate	09.727	5
	Isobutyl salicylate	09.750	5
	Isopentyl salicylate	09.751	5
	Benzyl salicylate	09.752	25
26	Diphenyl ether	04.035	5

(*): Except for fish and poultry.

3.2.2. Safety for the consumer

The safety for the consumer of the 27 compounds used as food flavourings has been already assessed by JECFA and EFSA as described in the former opinions of the FEEDAP Panel (see Table 3). All compounds are currently authorised in the EU as food flavourings without limitations.

Although deposition and residue studies of the compounds in farm animals are not available, the FEEDAP Panel considers that the use of these flavourings in animal feed would not appreciably increase the human exposure to these compounds. This is based on the expected extensive metabolism and excretion in target animals.

However, for some compounds belonging to CG 23, piperonal and vanillin, the FEEDAP Panel could not conclude on the safety for the consumer in the absence of ADME data in the target species. The applicant has provided a literature search to address the lack of data.

Piperonal

For piperonal, the EFSA FEEDAP Panel concluded in its previous opinion 'that the commonality of metabolic pathways in the laboratory animals, target species and humans has not been demonstrated. Consequently, no conclusion can be drawn on the safety for the consumer when this substance is used in animal nutrition' (EFSA FEEDAP Panel, 2012b).

In rats and mice, piperonal is extensively absorbed and metabolised, mainly by oxidation followed by conjugation reactions and rapidly excreted in urine (EFSA FEEDAP Panel, 2012b). However, in this opinion, it was concluded: 'For piperonal, the commonality of metabolic pathways in the laboratory animals, target species and humans has not been demonstrated'.

For the present assessment, the applicant submitted literature that supports the ability of target species to metabolise piperonal.

Piperonal, as an aromatic aldehyde, is presumed to be metabolised by aldehyde oxidases (AOX) (Garattini and Terao, 2012; Kurosaki et al., 2013). These enzymes are highly conserved across animal species, although the number of active AOX genes that encode for the respective isozymes varies among animal species (Garattini et al., 2009). As reviewed by these authors, all vertebrates express these enzymes, including ruminants, birds and fish. Kurosaki et al. (2013) also showed that several birds, including chickens, turkeys and ducks and several fish varieties express AOX. Thus, it can be assumed that all target species are able to oxidise piperonal to the respective carboxylic acid that is excreted after conjugation with endogenous compounds, mainly amino acids. Glycine is the principal conjugating amino acid in mammals (Smith et al., 2018), being ornithine and taurine the principal ones, respectively, in birds (Pan and Fouts, 1978) and in fish (Bend and James, 1978; Schlenk et al., 2008). Thus, piperonal is expected to be extensively metabolised and excreted in the target species, and residues of the parent compound and its metabolites are not expected.

Vanillin

For vanillin, the EFSA FEEDAP Panel concluded in its previous opinion that the lack of data on metabolism in poultry and fish precludes an assessment of consumer exposure from these sources (EFSA FEEDAP Panel, 2012b).

For the present assessment, the applicant submitted literature that supports the ability of poultry and fish to metabolise vanillin.

Vanillin is biotransformed by oxidation in several animal species to the respective carboxylic acid by aldehyde oxidases as demonstrated *in vitro* in hepatocytes of mouse, rat, monkey and human (Sahi et al., 2008) and in liver slices of guinea pigs (Panoutsopoulos and Beedham, 2005). It was experimentally demonstrated that aldehyde oxidases are also present and metabolically active in fish and birds. Vanillin was used as a standard substrate to evaluate the catalytic activity of these enzymes in rainbow trout (Aburas, 2014). Aldehyde oxidases were proven to be present in birds, including turkeys, chickens and ducks (Kurosaki et al., 2013). The carboxylic acid resultant from oxidation of vanillin can be conjugated with amino acids both in birds (Pan and Fouts, 1978) and fish (Bend and James, 1978; Schlenk et al., 2008) and excreted.

Wagenstaller and Buettner (2013) quantified by GC-MS vanillin in urine of human volunteers consuming their normal meals, except coffee for 2 days before sample collections. Data showed that vanillin was present in urine of humans, being 92% excreted as glucuronide conjugate (median concentration 33.1 µg/L). This study demonstrates that humans are normally exposed to vanillin through their diet. Thus, it is not expected that the residues eventually present in food products derived from animals exposed to vanillin as feed additive can appreciably increase the levels currently ingested.

Consequently, no safety concern would arise for the consumer from the use of these 27 compounds up to the highest levels considered safe for target animals.

3.2.3. Safety for the user

Regarding the safety for the user, for 5-methylhept-2-en-4-one [07.139] in CG 5, 5-methylfurfural [13.001] in CG 14 and 4-phenylbut-3-en-2-one [07.024] in CG 21, in the absence of studies, the FEEDAP Panel could not conclude on the safety for the users when handling the additives (EFSA FEEDAP Panel, 2016b, 2020a,b).

At the time of the previous assessment, the applicant produced the required safety data sheets (SDS), in which hazard for users were identified. The applicant states that 'exposure to such hazards shall be limited accordingly by the operator by taking the precautionary measures stipulated in the respective SDS: (i) use of protective material to avoid contact with skin and eyes for those additives for which hazards for skin and eye contact have been identified; (ii) operators should ensure adequate ventilation and workers shall use appropriate respiratory protectors to avoid inhalation of vapour or mist for those additives for which a hazard for respiratory exposure is recognised.'

The applicant did not provide experimental data on the safety for the user for any of the compounds following the requirements of the guidance on user safety. The applicant searched for existing evaluations by the European Chemical Agency (ECHA) or the Research Institute for Fragrance Materials (RIFM).

4-Phenylbut-3-en-2-one [07.024] has been pre-registered under REACH and the applicant provided information on the classification provided by companies.¹⁹

No new data were submitted on the safety for the user that would allow the FEEDAP Panel to change its previous conclusion.

3.2.3.1. Conclusions on safety for the user

Considering that there is no new evidence, the FEEDAP Panel reiterates that it is not in a position to conclude on the safety for the user of 5-methylhept-2-en-4-one [07.139] in CG 5, 5-methylfurfural [13.001] and 4-phenylbut-3-en-2-one [07.024].

3.2.4. Safety for the environment

In its previous assessments, the FEEDAP Panel concluded that the use of the 27 compounds under assessment in animal feed at the maximum safe level for the target species is considered safe for the environment. For 2-methyl-2-pentenoic acid [08.055] in CG 3, the FEEDAP Panel identified a potential concern for the use in marine aquaculture (sea cages) at the use levels considered safe for the target species and estimated a safe level of 0.05 mg/kg feed (EFSA FEEDAP Panel, 2015, 2016a).

To support the safety of use levels in feed higher than those considered safe for the environment in the previous assessments, the applicant provided experimental data, which would allow the FEEDAP Panel to revisit the conclusions on the safety for the environment for the 15 compounds under assessment and made a proposal to extrapolate to the remaining 12 compounds.

At the end of the tolerance trials, samples of faeces and urine were collected from animals from the control group and from the group administered with the maximum recommended level (1× MRD). For piglets, faecal samples (two animals per pen, all pens) and urine (one animal per pen, two pens per treatment) were collected at day 42. For cattle for fattening, faeces and pen manure samples were collected at day 42 from all animals and urine samples from two pens per treatment. For chickens for fattening, samples of excreta were collected at day 36 (from one animal per pen, all pens). The concentrations of the 15 components of the mixture were determined in all samples.

For each component, the fraction of the dose considered to be active (FA) was calculated as the ratio between the average concentration in manure at 1× MRD (corrected by the concentration in control) and the theoretical concentration of the compounds fed to the animals.

$$FA = \frac{[\text{Average } C_{\text{manure}} (1 \times \text{MRD}) - C_{\text{manure}}(\text{control})]}{\text{Theoretic } C_{\text{feed}}}$$

The concentration of the additives in manure from the control group and the group receiving 1 × MRD was calculated from the average concentrations of the additives in faeces and urine sample as follows:

$$C_{\text{manure}} = \frac{[(\text{Dung (kg)} \times \text{Conc Feces}) + (\text{Urine (kg)} \times \text{Conc Urine})]}{\text{Total manure (kg)}}$$

where piglet total manure is 84 kg (45 kg dung and 39 kg urine) and cattle for fattening total manure is 58 kg (40 kg dung and 18 kg urine).²⁰ The FEEDAP Panel notes that the metabolism study submitted does not comply with the provisions of the guidance (EFSA FEEDAP Panel, 2019). Particularly, the volume of excreta produced was not measured and default values (without a range of variability) were used to calculate the concentration in manure.

The concentrations in manure determined in samples taken at the end of the tolerance studies in poultry, pigs and cattle for fattening are summarised in Table 7.

¹⁹ Technical dossier/Annex_44_ECHA_2019_CG21_07_024. According to the classification provided by companies to ECHA in REACH registrations this substance may cause an allergic skin reaction. No hazard identified for inhalation exposure, dermal exposure (systemic effect) and eye exposure.

²⁰ Technical dossier/Supplementary information August 2020.

Table 7: Concentrations in manure of the 15 compounds tested in tolerance trials with 'milky-vanilla' mixture^(a)

CG	EU register name	FLAVIS No	Use level	Manure levels			Conclusion
				Poultry	Pigs	Cattle	
			mg/kg	% FA			
01	Butyric acid	08.005	125	–	–	–	Endogenously produced, extensively metabolised
01	Ethyl isovalerate	09.447	25	0.07%	0.03%	0.34%	Extensively metabolised
03	2-Methyl-2-pentenoic acid	08.055	5	0%	n.d.	n.d.	Extensively metabolised
05	6-Methylhept-5-en-2-one	07.015	4.5	1.40%	1.12%	0.38	Natural occurrence Extensively metabolised
05	Nonan-2-one	07.020	10	0.57%	0.77%	0.61%	Natural occurrence Extensively metabolised
05	5-Methylhept-2-en-4-one	07.139	5	0.99%	2.87%	0.65%	Extensively metabolised
09	Dodecano-1,5-lactone	10.008	25	0.34%	0.51%	0.03%	Extensively metabolised
14	5-Methylfurfural	13.001	5	2.80%	1.71%	0.68%	Natural occurrence Extensively metabolised
21	4-Phenylbut-3-en-2-one	07.024	5	1.93%	0.86%	0.42%	Extensively metabolised
23	4-Methoxybenzaldehyde (anisaldehyde)	05.015	25	0.61%	0.38%	0%	Extensively metabolised
23	Piperonal	05.016	5	2.42%	0.68%	0%	Natural occurrence Extensively metabolised
23	Vanillin	05.018	125	n.d.	n.d.%	0%	Natural occurrence Endogenously produced
23	Benzyl benzoate	09.727	5	11.24%	n.d.	0%	Metabolised (90%) in poultry, extensively metabolised in pigs and cattle for fattening Natural occurrence
23	Benzyl salicylate	09.752	25	2.27%	7.71%	1.25%	Extensively metabolised
26	Diphenyl ether	04.035	5	2.56%	1.92%	0.01%	Extensively metabolised

(a): The concentrations in manure were calculated from the concentrations determined in faeces and urine samples taken at the end of the tolerance studies in pigs and cattle for fattening and in excreta samples taken at the end of the tolerance study in poultry. The concentrations are expressed as the percentage of fraction of the dose considered to be active (%FA).

The analytical results expressed as %FA indicate that the majority of the compounds tested are extensively metabolised in the target species, the fraction in manure being <5% of the theoretical concentration fed to the animals. The data confirm the hypothesis made by the FEEDAP Panel that compounds belonging to CG 1 and 9 are extensively metabolised in the animals. For butyric acid [08.005], high concentrations in manure were found in samples from both the control and the treated groups, suggesting that butyric acid is endogenously produced. The applicant provided evidence from the literature to demonstrate the endogenous production of butyric acid in ruminants (Weller et al., 1969; Whitelaw et al., 1970; Bergman, 1990; den Besten et al., 2013; Mackie et al., 2013), pigs (Kien et al., 2000; Le, 2006) and poultry (Onrust et al., 2015). Butyric acid is fully metabolised via the tricarboxylic acid pathway and is not expected to be a concern for the environment.

A similar behaviour was observed for vanillin [05.018], which was found in manure in high concentrations, regardless of if it was added to feed or not. Vanillin is probably produced by the microbiota present in the rumen or in the gut starting from other sources, e.g. ferulic acid, anthocyanins, lignin and other constituents of the basal diet of animals (wheat, maize). In addition, the applicant provided evidence that vanillin is naturally occurring in plants at concentrations up to 31 mg/kg and in maize at concentrations up to 96 g/kg.²¹

²¹ Technical dossier/ EFSA_TT_M1_Annex_ERA_CG23/Annex_4_TNO_CG23_05_018.

Extensive metabolism in all species was also demonstrated for compounds belonging to CG 3, 5, 14, 21, 23 and 26, with the exception of benzyl benzoate [09.727] in poultry and benzyl salicylate [09.752] in pigs.

For 2-methyl-2-pentenoic acid [08.055] in CG 3, for which the natural occurrence could not be demonstrated, conclusions on the safety for the environment were based on a Phase II assessment made at the levels considered safe for the target species (EFSA FEEDAP Panel, 2016a). The new data provided indicate that extensive metabolism occurs in all target species; therefore, a Phase II assessment at the proposed use levels in feed is not required for these compounds.

For benzyl benzoate [09.727] in CG 23, the applicant provided evidence that it is naturally occurring in plants at concentrations up to 660 g/kg (in *Cinnamomum* species and in *Pistacia lentiscus*).²² For benzyl salicylate, the applicant provided evidence on the natural occurrence of salicylic acid [08.112] in European plants in concentrations higher than 25 mg/kg.²³

For diphenyl ether [04.035] belonging to CG 26, the FEEDAP Panel concluded that 'at a dose of 1 mg/kg these compounds are not expected to pose a risk for the environment. Their environmental consequences when used at a dose of 5 mg/kg complete feed are less certain and may result in predicted no-effect concentrations (PNECs) being exceeded in both water and soil compartments' (EFSA FEEDAP Panel, 2012c). The concentrations detected in manure of all target species indicate that the compound is extensively metabolised and a Phase II assessment at the proposed use levels in feed is not required.

For the compounds not tested in the tolerance trial, the applicant provided additional information on the natural occurrence and arguments for the read across from structurally related compounds tested in the tolerance trials, as summarised in Table 8. Based on the above (natural occurrence and/or extensive metabolism), a Phase II assessment is not required for these compounds at the proposed conditions of use.

Table 8: Conclusions for the 12 compounds non-tested in tolerance trials

CG	Product (EU register name)	FLAVIS No	Use level (mg/kg)	Conclusion
01	Ethyl butyrate	09.039	125	Read-across, extensive metabolism
	Ethyl isobutyrate	09.413	25	Read-across, extensive metabolism
	Methyl isovalerate	09.447	25	Read-across, extensive metabolism
05	Undecan-2-one	07.016	10	Natural occurrence
	Octan-2-one	07.019	10	Read-across, extensive metabolism
	Octan-3-one	07.062	10	Natural occurrence
	Tridecan-2-one	07.103	10	Natural occurrence
09	Tetradecano-1,5-lactone	10.016	5	Read-across, extensive metabolism
23	Anisyl alcohol	07.224	5	Natural occurrence, read-across
	Anisyl acetate	09.019	5	Read-across, metabolism
	Isobutyl salicylate	09.750	5	Read-across, metabolism
	Isopentyl salicylate	09.751	5	Read-across, metabolism

3.2.4.1. Conclusions on safety for the environment

For all the 27 compounds, the concentrations considered safe for the target species are unlikely to have detrimental effects on the environment.

4. Conclusions

The conclusions of the FEEDAP Panel on the maximum safe concentration of the 27 flavouring compounds in complete feed for all animal species are summarised as following:

²² Technical dossier/ EFSA_TT_M1_Annex_ERA_CG23/Annex_6_TNO_CG23_09_727.

²³ Technical dossier/ Annex_50_TNO_CG23_08_112_Salicylic acid.

CG	Product (EU register name)	FLAVIS No	All animal species (mg/kg complete feed)
01	Butyric acid	08.005	125
	Ethyl butyrate	09.039	125
	Ethyl isobutyrate	09.413	25
	Ethyl isovalerate	09.447	25
	Methyl isovalerate	09.462	5
03	2-Methyl-2-pentenoic acid	08.055	5
05	6-Methylhept-5-en-2-one	07.015	4.5
	Undecan-2-one	07.016	10
	Octan-2-one	07.019	10
	Nonan-2-one	07.020	10
	Octan-3-one	07.062	10
	Tridecan-2-one	07.103	10
	5-Methylhept-2-en-4-one	07.139	5
09	Dodecano-1,5-lactone	10.008	25
	Tetradecano-1,5-lactone	10.016	5
14	5-Methylfurfural	13.001	5
21	4-Phenylbut-3-en-2-one	07.024	5
23	Anisyl alcohol*	02.128	5
	4-Methoxybenzaldehyde (anisaldehyde)	05.015	25
	Piperonal	05.016	5
	Vanillin	05.018	125
	Anisyl acetate*	09.019	5
	Benzyl benzoate	09.727	5
	Isobutyl salicylate	09.750	5
	Isopentyl salicylate	09.751	5
	Benzyl salicylate	09.752	25
26	Diphenyl ether	04.035	5

(*): Except for fish and poultry.

No safety concern would arise for the consumer from the use of the 27 compounds up to the highest levels considered safe for target animals.

Considering that there is no new evidence, the FEEDAP Panel reiterates that it is not in a position to conclude on the safety for the user of 5-methylhept-2-en-4-one [07.139] in CG 5, 5-methylfurfural [13.001] and 4-phenylbut-3-en-2-one [07.024].

For all the 27 flavourings compounds, the concentrations considered safe for the target species are unlikely to have detrimental effects on the environment.

5. Documentation provided to EFSA/Chronology

Date	Event
30/11/2021	Dossier received by EFSA. Follow-up opinion linked to FAD (FAD-2010-0015/FAD-2010-0124/FAD-2010-0074/FAD-2010-0412/FAD-2010-0097/FAD-2010-0118/FAD-2010-0417/FAD-2010-0028/FAD-2010-0054) – 27 flavouring compounds to provide a Milky-Vanilla flavour for all animal species. Submitted by FEFANA asbl
14/02/2022	Reception mandate from the European Commission
02/03/2022	Application validated by EFSA – Start of the scientific assessment
10/05/2022	Request of supplementary information to the applicant in accordance with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. <i>Issues: safety for the target species</i>
27/07/2022	Reception of supplementary information from the applicant - Scientific assessment restarted
07/01/2023	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

- Aburas O, 2014. Investigation of aldehyde oxidase and xanthine oxidoreductases in rainbow trout (*Oncorhynchus mykiss*). PhD thesis. University of Huddersfield, July 2014, pp. 267.
- Bend JR and James MO, 1978. Xenobiotic metabolism in marine and freshwater species. In: DC Malins and JR Sargent (eds). *Biochemical and Biophysical Perspectives in Marine Biology*. 4. Academic Press, New York. pp. 126–188.
- Bergman EN, 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological reviews*, 70, 567–590. <https://doi.org/10.1152/physrev.1990.70.2.567>
- Den Besten G, Van Eunen K, Groen AK, Venema K, Reijngoud DJ and Bakker BM, 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, 54, 2325–2340. <https://doi.org/10.1194/jlr.R036012>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Scientific opinion on the safety and efficacy of aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers (chemical group 6) when used as flavourings for all animal species. *EFSA Journal* 2012;10(11):2966, 25 pp. <https://doi.org/10.2903/j.efsa.2012.2966>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Scientific Opinion on the safety and efficacy of benzyl alcohols, aldehydes, acids, esters and acetals (chemical group 23) when used as flavourings for all animal species. *EFSA Journal* 2012;10(7):2785, 30 pp. <https://doi.org/10.2903/j.efsa.2012.2785>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Scientific Opinion on the safety and efficacy of aromatic ethers including anisole derivatives (chemical group 26) when used as feed additives for all animal species. *EFSA Journal* 2012;10(5):2678, 19 pp. <https://doi.org/10.2903/j.efsa.2012.2678>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012d. Guidance for the preparation of dossiers for sensory additives. *EFSA Journal* 2012;10(1):2534, 26 pp. <https://doi.org/10.2903/j.efsa.2012.2534>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012e. Guidance on studies concerning the safety of use of the additive for users/workers. *EFSA Journal* 2012;10(1):2539, 5 pp. <https://doi.org/10.2903/j.efsa.2012.2539>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes (chemical group 01) when used as flavourings for all animal species. *EFSA Journal* 2013;11(4):3169, 35 pp. <https://doi.org/10.2903/j.efsa.2013.3169>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific opinion on the safety and efficacy of saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols belonging chemical group 5 when used as flavourings for all animal species. *EFSA Journal* 2015;13(11):4268, 21 pp. <https://doi.org/10.2903/j.efsa.2015.4268>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016a. Scientific opinion on the safety and efficacy of secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols from chemical group 8 when used as flavourings for all animal species. *EFSA Journal* 2016;14(6):4475, 26 pp. <https://doi.org/10.2903/j.efsa.2016.4475>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016b. Scientific opinion on the safety and efficacy of aliphatic and aromatic hydrocarbons (chemical Group 31) when used as flavourings for all animal species and categories. *EFSA Journal* 2016;14(1):4339, 17 pp. <https://doi.org/10.2903/j.efsa.2016.4339>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarres-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. *EFSA Journal* 2019;17(4):5648, 78 pp. <https://doi.org/10.2903/j.efsa.2019.5648>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Westendorf J, Gregoretti L, Manini P and Dusemund B, 2020a. Scientific Opinion on the safety and efficacy of oct-1-en-3-ol, pent-1-en-3-ol, oct-1-en-3-one, oct-1-en-3-yl acetate, isopulegol and 5-methylhept-2-en-4-one, belonging to chemical group 5 and of isopulegone and a-damascone belonging to chemical group 8 when used as flavourings for all animal species. *EFSA Journal* 2020;18(2):6002, 16 pp. <https://doi.org/10.2903/j.efsa.2020.6002>

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Westendorf J, Gregoret L, Manini P and Dusemund B, 2020b. Scientific Opinion on the safety and efficacy of 4-phenylbut-3-en-2-one and benzophenone belonging to chemical group 21 when used as flavouring compounds for all animal species. *EFSA Journal* 2020;18(2):6017, 12 pp. <https://doi.org/10.2903/j.efsa.2020.6017>
- Garattini and Terao, 2012. The role of aldehyde oxidase in drug metabolism. *Expert Opinion on Drug Metabolism & Toxicology*, 8, 487–503. <https://doi.org/10.1517/17425255.2012.663352>
- Garattini E, Fratelli M and Terao M, 2009. The mammalian aldehyde oxidase gene family. *Human Genomics*, 4, 119–130. <https://doi.org/10.1186/1479-7364-4-2-119>
- Kien CL, Chang JC and Cooper JR, 2000. Butyric acid is synthesized by piglets. *The Journal of Nutrition*, 130, 234–237. <https://doi.org/10.1093/jn/130.2.234>
- Kurosaki M, Bolis M, Fratelli M, Barzago MM, Pattini L, Perretta G, Terao M and Garattini E, 2013. Structure and evolution of vertebrate aldehyde oxidases: from gene duplication to gene suppression. *Cellular and Molecular Life Sciences*, 70, 1807–1830. <https://doi.org/10.1007/s00018-012-1229-5>
- Le DP, 2006. Odor from pig production: Its relation to diet. PhD Thesis. Wageningen Institute of Animal Science, Wageningen University and Research Centre, Wageningen, The Netherlands.
- Mackie RI, McSweeney CS and Aminov RI, 2013. *Rumen*. eLS. John Wiley & Sons, Ltd, Chichester. <https://doi.org/10.1002/9780470015902.a0000404.pub2>
- Onrust L, Ducatelle R, Van Driessche K, De Maesschalck C, Vermeulen K, Haesebrouck F, Eeckhaut V and Van Immerseel F, 2015. Steering endogenous butyrate production in the intestinal tract of broilers as a tool to improve gut health. *Frontiers in Veterinary Science*, 2, 75. <https://doi.org/10.3389/fvets.2015.00075>
- Pan and Fouts, 1978. Drug metabolism in birds. *Drug Metabolism Reviews*, 7, 1–253.
- Panoutsopoulos GI and Beedham C, 2005. Enzymatic oxidation of vanillin, isovanillin and protocatechuic aldehyde with freshly prepared Guinea pig liver slices. *Cellular Physiology and Biochemistry*, 15, 89–98. <https://doi.org/10.1159/000083641>
- Sahi J, Khan KK and Black CB, 2008. Aldehyde oxidase activity and inhibition in hepatocytes and cytosolic fractions from mouse, rat, monkey and human. *Drug Metabolism Letters*, 2, 176–183. <https://doi.org/10.2174/187231208785425818>
- Schlenk et al., 2008 In: *The Toxicology of Fishes*. Edited by Di Julio and Hinton, 2008.
- Smith RL, Cohen SM, Fukushima S, Gooderham NJ, Hecht SS, Guengerich FP, Rietjens IMCM, Bastaki M, Harman CL, McGowen MM and Taylor SV, 2018. The safety evaluation of food flavouring substances: the role of metabolic studies. *Toxicology Research*, 7, 618–646. <https://doi.org/10.1039/c7tx00254h>
- Wagenstaller M and Buettner A, 2013. Quantitative determination of common urinary odorants and their glucuronide conjugates in human urine. *Metabolites*, 3, 637–657. <https://doi.org/10.3390/metabo3030637>
- Weller RA, Pilgrim AF and Gray FV, 1969. Volatile fatty acid production in the rumen of the grazing sheep: its use as an indicator of pasture value. *The British Journal of Nutrition*, 23, 97–111.
- Whitelaw FG, Hyldgaard-Jensen J, Reid RS and Kay MG, 1970. Volatile fatty acid production in the rumen of cattle given an all-concentrate diet. *The British Journal of Nutrition*, 24, 179–195.

Abbreviations

ADG	Average daily gain
ADFI	Average daily feed intake
ANOVA	Analysis of variance
AOX	aldehyde oxidases
BW	body weight
CAS	Chemical Abstracts Service
CDG	chemically defined group
CG	chemical group
DM	dry matter
ECHA	European Chemical Agency
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FAF	EFSA Panel on Food Additives and Flavourings
FA	Active fraction
FAO	Food Agricultural Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FFAC	Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures)
FGE	food group evaluation

FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
GC-FID	gas chromatography-flame ionisation detector
GC-MS	Gas chromatography–mass spectrometry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MW	molecular weight
MRD	maximum recommended dose
MCH	mean corpuscular haemoglobin
NOAEL	no observed adverse effect level
PNEC	predicted no-effect concentration
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIFM	Research Institute for Fragrance Materials
SDS	Safety data sheet
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization