

ADOPTED: 6 December 2016

doi: 10.2903/j.efsa.2017.4671

Safety and efficacy of pyrazine derivatives including saturated ones belonging to chemical group 24 when used as flavourings for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Paul Brantom, Birgit Dusemund, Christer Hogstrand, Patrick Van Beelen, Johannes Westendorf, Lucilla Gregoretta, Paola Manini and Andrew Chesson

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 22 compounds belonging to chemical group 24 (pyrazine derivatives). They are currently authorised as flavours in food. The FEEDAP Panel concludes that: 2,3-diethylpyrazine [14.005], 2-ethyl-3-methylpyrazine [14.006], 5,6,7,8-tetrahydroquinoxaline [14.015], 2,3,5,6-tetramethylpyrazine [14.018], 2,3,5-trimethylpyrazine [14.019], 2,5-dimethylpyrazine [14.020], 2,6-dimethylpyrazine [14.021], 2-ethylpyrazine [14.022], 2-ethyl-3,5-dimethylpyrazine [14.024], 2,5 or 6-methoxy-3-methylpyrazine [14.025], 2-methylpyrazine [14.027], acetylpyrazine [14.032], 6,7-dihydro-5-methyl-5*H*-cyclopenta(b)pyrazine [14.037], 2-isobutyl-3-methoxypyrazine [14.043], 2-acetyl-3-ethylpyrazine [14.049], 2,3-dimethylpyrazine [14.050], 2,3-diethyl-5-methylpyrazine [14.056], 2-(*sec*-butyl)-3-methoxypyrazine [14.062], 3,(5- or 6-)-dimethyl-2-ethylpyrazine [14.100], 2-ethyl-3-methoxypyrazine [14.112] and 2-methoxy-3-methylpyrazine [14.126] are safe at the proposed maximum dose level (0.5 mg/kg complete feed) as feed for cattle, salmonids and non-food-producing animals, and at the proposed normal use level of 0.1 mg/kg complete feed for pigs and poultry; 5-methylquinoxaline [14.028] are safe only at concentrations below the proposed use levels (0.08 mg/kg complete feed for cattle, salmonids and non-food-producing animals, and 0.05 mg/kg complete feed for pigs and poultry). No safety concern would arise for the consumer from the use of these compounds up to the highest proposed level in feeds. Hazards for skin and eye contact, and respiratory exposure are recognised for the majority of the compounds under application. Most are classified as irritating to the respiratory system. The proposed maximum use levels in feed are unlikely to have detrimental effects on 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.

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: sensory additives, pyrazine derivatives, safety, chemical group 24

Requestor: European Commission

Question number: EFSA-Q-2010-01032

Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Coconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Lourdes Bastos M, Bories G, Coconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López Puente S, López-Alonso M, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dusemund B, Hogstrand C, Van Beelen P, Westendorf J, Gregoretto L, Manini P and Chesson A, 2017. Scientific opinion on the safety and efficacy of pyrazine derivatives including saturated ones belonging to chemical group 24 when used as flavourings for all animal species. *EFSA Journal* 2017;15(2):4671, 18 pp. doi:10.2903/j.efsa.2017.4671

ISSN: 1831-4732

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs License](https://creativecommons.org/licenses/by-nd/4.0/), which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference.....	4
1.2. Additional information.....	4
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Characterisation.....	5
3.1.1. Characterisation of the flavouring additives.....	5
3.1.2. Stability.....	8
3.1.3. Conditions of use.....	8
3.2. Safety.....	8
3.2.1. Absorption, distribution, metabolism and excretion (ADME).....	9
3.2.2. Toxicological studies.....	9
3.2.3. Safety for the target species.....	9
3.2.3.1. Conclusions on safety for the target species.....	11
3.2.4. Safety for the consumer.....	11
3.2.5. Safety for the user.....	11
3.2.6. Safety for the environment.....	11
3.2.6.1. Conclusions on safety for the environment.....	14
3.3. Efficacy.....	14
4. Conclusions.....	14
Documentation provided to EFSA.....	14
References.....	14
Abbreviations.....	16
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Chemically defined flavourings from Chemical group 24 – Pyrazine derivatives.....	18

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 22 substances belonging to chemical group (CG) 24 (2,3-diethylpyrazine, 2-ethyl-3-methylpyrazine, 5,6,7,8-tetrahydroquinoxaline, 2,3,5,6-tetramethylpyrazine, 2,3,5-trimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,5 or 6-methoxy-3-methylpyrazine, 2-methylpyrazine, 5-methylquinoxaline, acetylpyrazine, 5H-5-methyl-6,7-dihydrocyclopenta(b)pyrazine (herein referred to as 6,7-dihydro-5-methyl-5H-cyclopenta(b)pyrazine), 2-isobutyl-3-methoxypyrazine, 2-acetyl-3-ethylpyrazine, 2,3-dimethylpyrazine, diethyl-5-methylpyrazine (herein referred to as 2,3-diethyl-5-methylpyrazine), 2-(sec-butyl)-3-methoxypyrazine, 2-ethyl-3,(5or6)dimethylpyrazine (herein referred to as 3,(5 or 6)-2-ethyl-dimethylpyrazine), 2-ethyl-3-methoxypyrazine, 2-methoxy-3-methylpyrazine), when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). CG 24 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000³ as 'Pyrazine derivatives'.

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 10 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 2,3-diethylpyrazine [The EU Flavour Information System (FLAVIS) Number 14.005], 2-ethyl-3-methylpyrazine [14.006], 5,6,7,8-tetrahydroquinoxaline [14.015], 2,3,5,6-tetramethylpyrazine [14.018], 2,3,5-trimethylpyrazine [14.019], 2,5-dimethylpyrazine [14.020], 2,6-dimethylpyrazine [14.021], 2-ethylpyrazine [14.022], 2-ethyl-3,5-dimethylpyrazine [14.024], 2,5 or 6-methoxy-3-methylpyrazine [14.025], 2-methylpyrazine [14.027], 5-methylquinoxaline [14.028], acetylpyrazine [14.032], 6,7-dihydro-5-methyl-5H-cyclopenta(b)pyrazine [14.037], 2-isobutyl-3-methoxypyrazine [14.043], 2-acetyl-3-ethylpyrazine [14.049], 2,3-dimethylpyrazine [14.050], 2,3-diethyl-5-methylpyrazine [14.056], 2-(sec-butyl)-3-methoxypyrazine [14.062], 3,(5- or 6)-dimethyl-2-ethylpyrazine [14.100], 2-ethyl-3-methoxypyrazine [14.112], 2-methoxy-3-methylpyrazine [14.126] when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

All 22 substances except 2-ethyl-3-methoxypyrazine [14.112] and 2-methoxy-3-methylpyrazine [14.126] 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, 2002a,b) and were considered safe for use in food. No acceptable daily intake (ADI) values were established.

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.

Subsequently, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) assessed all compounds and concluded that they do not give rise to safety concerns when used as flavour in food (EFSA 2008a, EFSA CEF Panel, 2011a).

All 22 compounds are all currently listed in the European Union (EU) database of flavouring substances⁵ 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 429/2008⁶ 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 dossier⁷ in support of the authorisation request for the use of the compounds belonging to CG 24 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 'pyrazine derivatives' 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 pyrazine derivatives is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

3.1. Characterisation

3.1.1. Characterisation of the flavouring additives

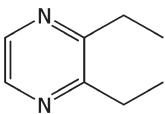
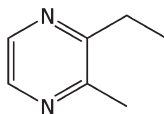
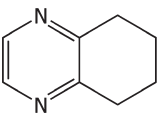
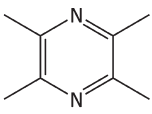
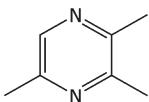
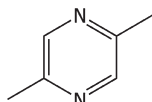
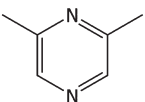
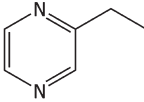
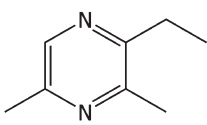
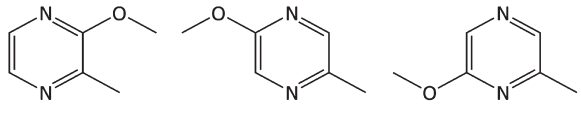
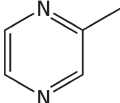
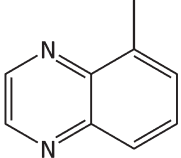
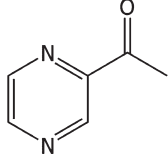
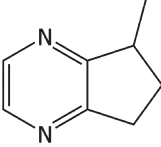
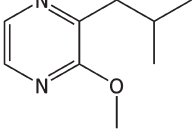
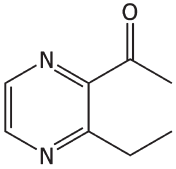
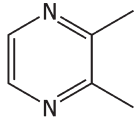
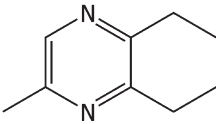
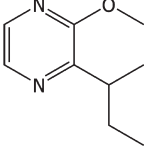
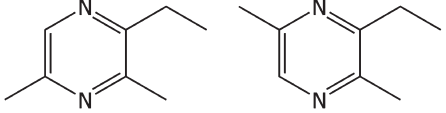
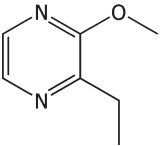
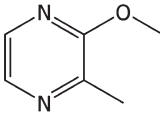
The molecular structures of the 22 additives under application are shown in Figure 1 and their physicochemical characteristics in Table 1.

⁵ Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

⁶ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

⁷ FEED dossier reference: FAD-2010-0053.

⁸ The full report is available on the EURL website <https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0053.pdf>

2,3-Diethylpyrazine [14.005] 	2-Ethyl-3-methylpyrazine [14.006] 	5,6,7,8-Tetrahydro quinoxaline [14.015] 	2,3,5,6-Tetramethyl pyrazine [14.018] 
2,3,5-Trimethylpyrazine [14.019] 	2,5-Dimethylpyrazine [14.020] 	2,6-Dimethylpyrazine [14.021] 	2-Ethylpyrazine [14.022] 
2-Ethyl-3,5-dimethyl pyrazine [14.024] 	2,5 or 6-Methoxy-3-methylpyrazine [14.025]  2-Methoxy-3-methylpyrazine 6-Methoxy-3-methylpyrazine 6-Methoxy-3-methylpyrazine	2-Methylpyrazine [14.027] 	
5-Methylquinoxaline [14.028] 	Acetylpyrazine [14.032] 	6,7-Dihydro-5-methyl-5H- cyclopenta(b)pyrazine ^(a) [14.037] 	2-Isobutyl-3-methoxy pyrazine [14.043] 
2-Acetyl-3-ethylpyrazine [14.049] 	2,3-Dimethylpyrazine [14.050] 	2,3-Diethyl-5- methylpyrazine ^(b) [14.056] 	2-(sec-Butyl)-3- methoxypyrazine [14.062] 
3,(5- or 6-)-Dimethyl-2-ethyl-pyrazine ^(c) [14.100]  2-Ethyl-3,5-dimethylpyrazine 2-Ethyl-3,6-dimethylpyrazine	2-Ethyl-3-methoxypyrazine [14.112] 	2-Methoxy-3- methylpyrazine [14.126] 	

(a): In the technical dossier, the compound is identified as 5H-5-methyl-6,7-dihydrocyclopenta(b) pyrazine.

(b): In the technical dossier, the compound is identified as diethyl-5-methylpyrazine.

(c): In the technical dossier, the compound is identified as 2-ethyl-3,(5 or 6)dimethylpyrazine.

Figure 1: Molecular structures and [FLAVIS numbers] of the 22 flavouring compounds under assessment

Table 1: Chemical Abstracts Service (CAS) and FLAVIS numbers and some characteristics of the 22 flavouring compounds under assessment

EU Register name	CAS No	FLAVIS No	Molecular formula	Molecular weight	Physical state	Log K_{ow} ^(a)
2,3-Diethylpyrazine	15707-24-1	14.005	C ₈ H ₁₂ N ₂	136.2	Liquid	1.51
2-Ethyl-3-methylpyrazine	15707-23-0	14.006	C ₇ H ₁₀ N ₂	122.17	Liquid	1.07
5,6,7,8-Tetrahydroquinoxaline	34413-35-9	14.015	C ₈ H ₁₀ N ₂	134.18	Solid	1.41
2,3,5,6-Tetramethylpyrazine	1124-11-4	14.018	C ₈ H ₁₂ N ₂	136.2	Solid	1.82
2,3,5-Trimethylpyrazine	14667-55-1	14.019	C ₇ H ₁₀ N ₂	122.17	Liquid	0.95
2,5-Dimethylpyrazine	123-32-0	14.020	C ₆ H ₈ N ₂	108.14	Liquid	0.63
2,6-Dimethylpyrazine	108-50-9	14.021	C ₆ H ₈ N ₂	108.14	Solid	0.54
2-Ethylpyrazine	13925-00-3	14.022	C ₆ H ₈ N ₂	108.14	Liquid	0.69
2-Ethyl-3,5-dimethylpyrazine	13925-07-0 ^(b)	14.024	C ₈ H ₁₂ N ₂	136.2	Liquid	1.63
2,5- or 6-Methoxy-3-methylpyrazine ^(c)	63450-30-6	14.025	C ₆ H ₈ ON ₂	124.14	Liquid	-0.28
2-Methylpyrazine	109-08-0	14.027	C ₅ H ₆ N ₂	94.12	Liquid	0.21
5-Methylquinoxaline	13708-12-8	14.028	C ₉ H ₈ N ₂	144.18	Liquid	2.04
Acetylpyrazine	22047-25-2	14.032	C ₆ H ₆ ON ₂	122.13	Solid	0.2
6,7-Dihydro-5-methyl-5H-cyclopenta(b)pyrazine	23747-48-0	14.037	C ₈ H ₁₀ N ₂	134.18	Liquid	1.83
2-Isobutyl-3-methoxypyrazine	24683-00-9	14.043	C ₉ H ₁₄ ON ₂	166.22	Liquid	2.86
2-Acetyl-3-ethylpyrazine	32974-92-8	14.049	C ₈ H ₁₀ ON ₂	150.18	Liquid	1.15
2,3-Dimethylpyrazine	5910-89-4	14.050	C ₆ H ₈ N ₂	108.14	Liquid	0.54
2,3-Diethyl-5-methylpyrazine	18138-04-0	14.056	C ₉ H ₁₄ N ₂	150.22	Liquid	1.95
2-(<i>sec</i> -Butyl)-3-methoxypyrazine	24168-70-5	14.062	C ₉ H ₁₄ ON ₂	166.22	Liquid	1.92
3,(5- or 6-)-Dimethyl-2-ethylpyrazine ^(d)	27043-05-6	14.100	C ₈ H ₁₂ N ₂	136.2	Liquid	2.07*
2-Ethyl-3-methoxypyrazine	25680-58-4	14.112	C ₇ H ₁₀ N ₂ O	132.14	Liquid	1.80
2-Methoxy-3-methylpyrazine	2847-30-5	14.126	C ₆ H ₈ N ₂ O	124.14	Liquid	1.24

EU: European Union; CAS No: Chemical Abstract Service number.; FLAVIS number: EU Flavour Information System number.

(a): Logarithm of octanol–water partition coefficient.

(b): Three CAS numbers have been used to identify 2-ethyl-3,5-dimethylpyrazine, two refer to 2-ethyl-3,5-dimethylpyrazine (CAS Nos 13925-07-0 and 55031-15-7) and one to mixture of isomers 2-ethyl-3,(5 or 6)-dimethylpyrazine (CAS No 27043-05-6). They are all relevant to the assessment of this compound.

(c): Mixture of three positional methoxy-isomers: 2-methoxy-3-methylpyrazine (75–85%); 2-methoxy-5-methylpyrazine (15–25%) and 2-methoxy-6-methylpyrazine (1–2%) (sum 97%). The CAS number applies to all three isomers. Different CAS numbers are associated to individual isomers: 2-methoxy-3-methylpyrazine (CAS No 2847-30-5), 2-methoxy-5-methylpyrazine (CAS No 2882-22-6) and 5-methoxy-3-methylpyrazine (CAS No 2882-21-5).

(d): The additive is a mixture of the two isomers. The CAS number applies to the mixture. A different CAS number (55031-15-7) reported in the dossier refers to 2-ethyl-3,5-dimethylpyrazine. See also (2).

*: Generated from Epi-Suite 4.01.

These substances are produced by chemical synthesis. Several routes of synthesis are available and described in the dossier.⁹

Batch-to-batch variation data were provided for five batches of each additive with the exception of 2,3-diethylpyrazine (one batch available due to the low use volume), 2-ethylpyrazine and 2-acetyl-3-ethylpyrazine (three batches), diethyl-5-methyl pyrazine and 2-(*sec*-butyl)-3-methoxypyrazine (four batches).¹⁰ The content of the active substance for all compounds exceeded the JECFA specifications (Table 2).

⁹ Technical dossier/Section II.

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

Table 2: Identity of the substances and data on purity

EU Register name	FLAVIS No	JECFA specification minimum % ^(a)	Assay %	
			Average	Range
2,3-Diethylpyrazine	14.005	> 97	99.2 ^(b)	–
2-Ethyl-3-methylpyrazine	14.006	> 97*	99.9	99.5–100
5,6,7,8-Tetrahydroquinoxaline	14.015	> 98	99.3	99.0–99.7
2,3,5,6-Tetramethylpyrazine	14.018	> 95	99.7	98.5–100
2,3,5-Trimethylpyrazine	14.019	> 98	99.6	98.2–100
2,5-Dimethylpyrazine	14.020	> 98*	99.6	99.2–99.9
2,6-Dimethylpyrazine	14.021	> 98*	99.7	99.4–100
2-Ethylpyrazine	14.022	> 98	99.4	99.3–99.6
2-Ethyl-3,5-dimethylpyrazine	14.024	> 95	99.9	99.6–100
2,5 or 6-Methoxy-3-methylpyrazine	14.025	> 97*	100.0	99.9–100
2-Methylpyrazine	14.027	> 98	99.8	99.6–100
5-Methylquinoxaline	14.028	> 98	100.0	99.9–100
Acetylpyrazine	14.032	> 99	99.9	99.8–100
6,7-Dihydro-5-methyl-5 <i>H</i> -cyclopenta(b)pyrazine	14.037	> 97	98.4	98.3–98.8
2-Isobutyl-3-methoxypyrazine	14.043	> 95	99.8	99.6–100
2-Acetyl-3-ethylpyrazine	14.049	> 98	99.8	99.6–100
2,3-Dimethylpyrazine	14.050	> 95*	99.8	99.6–100
2,3-Diethyl-5-methylpyrazine	14.056	> 98	99.6	99.4–99.6
2-(<i>sec</i> -Butyl)-3-methoxypyrazine	14.062	> 99	99.6	99.0–100
3,(5- or 6-)-Dimethyl-2-ethylpyrazine	14.100	> 95	99.9*	99.9–100
2-Ethyl-3-methoxypyrazine	14.112	> 99	99.8	99.5–99.9
2-Methoxy-3-methylpyrazine	14.126	> 97	99.3 ^(c)	97.4–100

FLAVIS number: EU Flavour Information System number.

(a): FAO, 2006.

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

(c): Specifications reflect commercial product.

*: Sum of isomers.

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 is at least 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 22 additives in feed for all animal species without withdrawal. For all 22 additives, the applicant proposes a normal use level of 0.1 mg/kg feed and a high use level of 0.5 mg/kg.

3.2. Safety

The assessment of safety is based on the highest use level proposed by the applicant (0.5 mg/kg complete feed).

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

Compounds belonging to CG 24 are absorbed from the gastrointestinal tract and share common pathways of metabolism: (i) oxidation of the side-chain(s) of alkyl-, alicyclic- and alkylaryl substituted pyrazine derivatives, (ii) oxidation of methylpyrazines to the corresponding pyrazine-2-carboxylic acids (iii) hydroxylation of the pyrazine ring, (iv) reduction of the ketone functional group of acylated pyrazines to the corresponding secondary alcohol and (v) conjugation of products of oxidative metabolism with glycine, glucuronic acid or sulfate, and glucuronidation of secondary alcohols (WHO, 2002a,b, FAS 48).

Metabolic studies of pyrazine derivatives mainly performed in the rat after oral administration showed that rats dosed individually with a number of compounds under assessment have an efficient excretion mechanism for pyrazine derivatives at 100 mg/kg body weight (bw). About 90% of 2-methylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine were excreted within 24 h as the corresponding pyrazine-2-carboxylic acid derivative. This acid was mainly eliminated unconjugated, with 10–15% excreted as a glycine conjugate. For 2,3-dimethylpyrazine, due to the steric hindrance of the methyl groups, only 10–15% was oxidised to 2-methylpyrazine-3-carboxylic acid. 2,3-Dimethyl-5-hydroxypyrazine is the main metabolite, attaining about 40% of the administered dose and eliminated as a conjugate. Also in rats, 2-isobutyl-3-methoxypyrazine was eliminated in urine mainly as the conjugate of the O-demethylated metabolite. A minor metabolite 2-methoxy-3-(2-carboxypropyl)pyrazine was also identified resulting from the oxidation of the aliphatic side-chain (Hawksworth and Scheline, 1975).

Studies of metabolism of pyrazine derivatives in animals other than rodents are lacking in the scientific literature. However, the enzymes involved in the biotransformation pathways of these compounds are present in all target species. Alkyl groups of pyrazine derivatives are oxidised mainly by P450 type enzymes to form the corresponding alcohols or carboxylic acids; the ring is hydroxylated by molybdenum-containing oxidases of the xanthine oxidase type (Müller and Rappert, 2010). The CYP450 monooxygenase families are present and have been characterised in a number of food-producing animals, including ruminants, horses, pigs, (Nebbia et al., 2003; Ioannides, 2006; Fink-Gremmels, 2008), fish (Wolf and Wolfe, 2005) and birds (Blevins et al., 2012). The molybdenum hydroxylases are also present in several animal species (Morikawa et al., 1997; Hainline and Rajagopalan, 2013). Cytosolic carbonyl reductases that reduce ketones to secondary alcohols were characterised in the liver and kidney of several animal species, namely chicken, rabbit and sheep, as reviewed by Felsted and Bachur (1980). Thus, it is expected that the acyl-pyrazine derivatives can be reduced and subsequently conjugated for excretion. Phase II conjugation via glucuronidation, sulfation or addition of glycine has been reported to occur in mammals although the predominance of one pathway over another varies among animal species (Gupta, 2007). All target species, also carry out conjugation reactions with sulfate and glucuronic acid (Watkins and Klaassen, 1986; James, 1987; Gusson et al., 2006), producing water-soluble derivatives that are eliminated in urine. The FEEDAP Panel notes that for feline species the capacity for conjugation is limited (Court, 2013).

3.2.2. Toxicological studies

Subchronic, repeated-dose studies, with multiple doses tested could not be found for any of the compounds under assessment.

Summaries of toxicological data on single dose level studies are available for 2,3-diethylpyrazine [14.005], 2-ethyl-3-methylpyrazine [14.006], 2-ethyl-3,5-dimethylpyrazine [14.024], 2,5 or 6-methoxy-3-methylpyrazine [14.025] and 5-methylquinoxaline [14.028] (Posternak et al., 1969), and acetylpyrazine [14.032] (Posternak et al., 1975). Secondary references referred to repeated dose toxicity studies (90 days, one dose tested) in rat performed with 5,6,7,8-tetrahydroquinoxaline [14.015] (Oser, 1970), 2,3,5,6-tetramethylpyrazine [14.018] (Oser, 1969a), 2,3,5-trimethylpyrazine [14.019] (Oser, 1969b), 2,6-dimethylpyrazine [14.021], (Oser, 1969d), 3,(5- or 6-)-dimethyl-2-ethylpyrazine [14.100] (Oser, 1969c) and 6,7-dihydro-5-methyl-5*H*-cyclopenta(b)pyrazine [14.037] (Wheldon and Krajceman, 1967). Study reports for these compounds are not available and the FEEDAP Panel is unable to confirm the no observed adverse effect levels (NOAELs) derived.

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 CEF Panel, 2011a,b) ranges from 0.1 to 100 µg/person per day, corresponding to 0.005 to 4.6 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 ($\mu\text{g}/\text{kg bw}^{0.75}$ per day) ^(a)	Target animal exposure ($\mu\text{g}/\text{kg bw}^{0.75}$ per day)		
			Salmon	Piglet	Dairy cow
2,3-Diethylpyrazine	0.5	0.07	11.8	52.6	77.7
2-Ethyl-3-methylpyrazine	0.5	3.34	11.8	52.6	77.7
5,6,7,8-Tetrahydroquinoxaline	0.5	0.37	11.8	52.6	77.7
2,3,5,6-Tetramethylpyrazine	0.5	0.31	11.8	52.6	77.7
2,3,5-Trimethylpyrazine	0.5	4.46	11.8	52.6	77.7
2,5-Dimethylpyrazine	0.5	0.88	11.8	52.6	77.7
2,6-Dimethylpyrazine	0.5	0.06	11.8	52.6	77.7
2-Ethylpyrazine	0.5	0.10	11.8	52.6	77.7
2-Ethyl-3,5-dimethylpyrazine	0.5	0.06	11.8	52.6	77.7
2,5 or 6-Methoxy-3-methylpyrazine	0.5	0.70 ^(b)	11.8	52.6	77.7
2-Methylpyrazine	0.5	0.79	11.8	52.6	77.7
5-Methylquinoxaline	0.5	1.02	11.8	52.6	77.7
Acetylpyrazine	0.5	0.56	11.8	52.6	77.7
6,7-Dihydro-5-methyl-5H-cyclopenta(b)pyrazine	0.5	0.18	11.8	52.6	77.7
2-Isobutyl-3-methoxypyrazine	0.5	4.92	11.8	52.6	77.7
2-Acetyl-3-ethylpyrazine	0.5	0.03	11.8	52.6	77.7
2,3-Dimethylpyrazine	0.5	0.65	11.8	52.6	77.7
Diethyl-5-methylpyrazine	0.5	0.05	11.8	52.6	77.7
2-(sec-Butyl)-3-methoxypyrazine	0.5	0.04	11.8	52.6	77.7
3,(5- or 6-)-Dimethyl-2-ethylpyrazine	0.5	1.76	11.8	52.6	77.7
2-Ethyl-3-methoxypyrazine	0.5	0.05 ^(b)	11.8	52.6	77.7
2-Methoxy-3-methylpyrazine	0.5	0.70	11.8	52.6	77.7

bw: body weight.

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

(b): EU figures not available (exposure based on US intake figures).

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. However, adequate subchronic, repeated-dose studies performed with the additives under assessment were not available. Therefore, the threshold of toxicological concern (TTC) approach was followed to derive the maximum safe feed concentration (EFSA FEEDAP Panel, 2012a).

All compounds except 5-methylquinoxaline belong to the Cramer Class II. The calculated safe use level for these compounds is 0.5 mg/kg complete feed for cattle, salmonids and non-food-producing animals, and 0.3 mg/kg complete feed for pigs and poultry. 5-Methylquinoxaline (Cramer Class III) is safe at 0.0 mg/kg complete feed for cattle, salmonids and non-food-producing animals, and 0.05 mg/kg complete feed for pigs and poultry.

3.2.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that:

- 2,3-diethylpyrazine [14.005], 2-ethyl-3-methylpyrazine [14.006], 5,6,7,8-tetrahydroquinoxaline [14.015], 2,3,5,6-tetramethylpyrazine [14.018], 2,3,5-trimethylpyrazine [14.019], 2,5-dimethylpyrazine [14.020], 2,6-dimethylpyrazine [14.021], 2-ethylpyrazine [14.022], 2-ethyl-3,5-dimethylpyrazine [14.024], 2,5 or 6-methoxy-3-methylpyrazine [14.025], 2-methylpyrazine [14.027], acetylpyrazine [14.032], 6,7-dihydro-5-methyl-5*H*-cyclopenta(b)pyrazine [14.037], 2-isobutyl-3-methoxypyrazine [14.043], 2-acetyl-3-ethylpyrazine [14.049], 2,3-dimethylpyrazine [14.050], 2,3-diethyl-5-methylpyrazine [14.056], 2-(*sec*-butyl)-3-methoxypyrazine [14.062], 3,(5- or 6-)-dimethyl-2-ethylpyrazine [14.100], 2-ethyl-3-methoxypyrazine [14.112] and 2-methoxy-3-methylpyrazine [14.126] are safe at the proposed maximum dose level (0.5 mg/kg complete feed) for cattle, salmonids and non-food-producing animals, and at the proposed normal use level of 0.1 mg/kg complete feed for pigs and poultry;
- 5-methylquinoxaline [14.028] is safe only at concentrations below the proposed use levels (0.08 mg/kg complete feed for cattle, salmonids and non-food-producing animals, and 0.05 mg/kg complete feed for pigs and poultry).

3.2.4. Safety for the consumer

The safety for the consumer of the compounds in CG 24, used as food flavours, has already been assessed by JECFA (WHO, 2002a,b) and EFSA (EFSA 2008a, EFSA CEF Panel, 2011a). All these compounds are presently authorised as food flavourings without limitations.⁵

Given the proposed use levels of CG 24 compounds to be applied in feed, the expected metabolism and excretion in target animals (see Section 3.2.1), the FEEDAP Panel considers that the possible residues in food derived from animals fed with these flavourings would not appreciably increase the human intake levels of these compounds. Consequently, no safety concern would arise for the consumer from the use of these 22 compounds up to the highest safe level in feeds.

3.2.5. Safety for the user

No specific data on the safety for the user were provided. In the material safety data sheets,¹¹ hazards for skin and eye contact and respiratory exposure are recognised for the majority of the compounds under application. Most are classified as irritating to the respiratory system.

3.2.6. Safety for the environment

The additions of naturally occurring substances that will not result in a substantial increase in the concentration in the environment are exempt from further assessment. Examination of the published literature shows that this applies to 14 substances, namely 2-ethyl-3-methylpyrazine [14.006], 2,3,5,6-tetramethylpyrazine [14.018], 2,3,5-trimethylpyrazine [14.019], 2,5-dimethylpyrazine [14.020], 2,6-dimethylpyrazine [14.021], 2-ethylpyrazine [14.022], 2-ethyl-3,5-dimethylpyrazine [14.024], 2,5 or 6-methoxy-3-methylpyrazine [14.025], 2-methylpyrazine [14.027], acetylpyrazine [14.032], 6,7-dihydro-5-methyl-5*H*-cyclopenta(b)pyrazine [14.037], 2-isobutyl-3-methoxypyrazine [14.043], 2,3-dimethylpyrazine [14.050] and 3,(5- or 6-)-dimethyl-2-ethylpyrazine [14.100], which occur in the environment at levels above the application rate of 0.5 mg/kg feed (data taken from the Netherlands Organisation for Applied Scientific Research (TNO) database Volatile Compounds in Food ver. 14.1; Burdock, 2003).¹²

The other eight compounds, namely 2,3-diethylpyrazine [14.005], 5,6,7,8-tetrahydroquinoxaline [14.015], 5-methylquinoxaline [14.028], 2-acetyl-3-ethylpyrazine [14.049], 2,3-diethyl-5-methylpyrazine [14.056], 2-(*sec*-butyl)-3-methoxypyrazine [14.062], 2-ethyl-3-methoxypyrazine [14.112] and 2-methoxy-3-methylpyrazine [14.126], could not be shown to occur in the environment at levels above the application rate of 0.5 mg/kg feed. For these compounds, the predicted environmental concentration for soil (PEC_{soil}) was calculated based on the use rate (Table 4) and compared with the trigger values for compartments set in the phase I of the EFSA guidance on environmental risk assessment for feed additives (EFSA, 2008b).

¹¹ Technical dossier/Section II/Annex II.3.

¹² Technical dossier/Supplementary information June 2011.

Table 4: Predicted environmental concentration (PEC) values of the eight flavourings of CG 24 under assessment (calculated for lamb manure)

EU Register name	CAS No	Dose mg/kg	PEC _{soil} (µg/kg)	PEC _{porewater} (µg/L)	PEC _{surfacewater} (µg/L)
2,3-Diethylpyrazine	15707-24-1	0.5	10.7	15.5	5.2
5,6,7,8-Tetrahydroquinoxaline	34413-35-9	0.5	10.7	1.5	0.5
5-Methylquinoxaline	13708-12-8	0.5	10.7	5.2	1.7
2-Acetyl-3-ethylpyrazine	32974-92-8	0.5	10.7	12.7	4.2
2,3-Diethyl-5-methylpyrazine	18138-04-0	0.5	10.7	6.3	2.1
2-(sec-Butyl)-3-methoxypyrazine	24168-70-5	0.5	10.7	2.7	0.9
2-Ethyl-3-methoxypyrazine	25680-58-4	0.5	10.7	8.7	2.9
2-Methoxy-3-methylpyrazine	2847-30-5	0.5	10.7	25.1	8.4

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

The PEC_{soil} values are above the threshold of 10 µg/kg (EFSA, 2008b). The PEC for pore water (PEC_{porewater}) 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 5).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 5: Physicochemical properties predicted by EPIWEB 4.1 for the eight flavourings of CG 24 under assessment

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)
2,3-Diethylpyrazine	15707-24-1	15	136.20	103.0	4,458	32.4
5,6,7,8-Tetrahydroquinoxaline	34413-35-9	15	134.20	12.4	2,091	391
5-Methylquinoxaline	13708-12-8	13	144.18	0.7	1,456	110
2-Acetyl-3-ethylpyrazine	32974-92-8	14	150.18	2.2	20,720	41
2,3-Diethyl-5-methylpyrazine	18138-04-0	20	150.23	73.1	1,636	89
2-(sec-Butyl)-3-methoxypyrazine	24168-70-5	17	166.22	4.7	230	220
2-Ethyl-3-methoxypyrazine	25680-58-4	15	138.17	23.2	2,474	63
2-Methoxy-3-methylpyrazine	2847-30-5	14	124.14	77.9	8,457	17

EU: European Union; CAS No: Chemical Abstracts Service number.

(a): DT₅₀, half-life of the additive (EPIWB 4.1.BioWin4.1).

(b): K_{oc} , organic carbon sorption constant (EPIWB 4.1.KocWin2.0).

The half-life (DT₅₀) was calculated using BioWin4.1 (Ultimate Survey Model), which gives a rating number. This rating number r was translated into a half-life using the formula by Arnot et al. (2005):

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

This is the general regression used to derive estimates of aerobic environmental biodegradation half-lives from BioWin4.1 model output.

¹³ Available online: <http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm>

For the eight substances, the calculated predicted concentrations for groundwater (PEC_{porewater}) are above 0.1 µg/L and for soil (PEC_{soil}) above 10 µg/kg (Table 4). Therefore, they are subject 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 (EC₅₀) or lethal concentration (LC₅₀) for earthworms, fish, green algae and daphnids from the SMILES notation of the substance. The predicted no effect concentration (PNEC) for terrestrial environment (PNEC_{soil}) was determined by dividing the LC₅₀ earthworm by an uncertainty factor (UF) of 1,000. The corresponding PNEC for aquatic compartment (PNEC_{aquatic}) was derived from the lowest toxicity value for freshwater environment by applying an UF of 1,000.

Table 6: Phase II environmental risk assessment of soil and aquatic compartments for CG 24 compounds used as feed additives for terrestrial farm animals (exposure and effect data were modelled using EPIWEB 4.1 and ECOSAR 1.11)

EU Register name Soil	LC ₅₀ ^(a) Earthworm (mg/kg)			PNEC _{soil} (µg/kg)	PEC _{soil} (µg/kg)	PEC _{soil} /PNEC _{soil}
	2,3-Diethylpyrazine	236			236	10.7
5,6,7,8-Tetrahydroquinoxaline	238			238	10.7	0.04
5-Methylquinoxaline	272			272	10.7	0.04
2-Acetyl-3-ethylpyrazine	360			360	10.7	0.03
2,3-Diethyl-5-methylpyrazine	228			228	10.7	0.05
2-(sec-Butyl)-3-methoxy-pyrazine	235			235	10.7	0.05
2-Ethyl-3-methoxy-pyrazine	243			243	10.7	0.04
2-Methoxy-3-methylpyrazine	245			245	10.7	0.04
Aquatic	LC ₅₀ 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 _{sw}
	2,3-Diethylpyrazine	108	61	46	46	5.2
5,6,7,8-Tetrahydroquinoxaline	134	75	54	54	0.5	0.01
5-Methylquinoxaline	238	131	86	86	1.7	0.02
2-Acetyl-3-ethylpyrazine	1,974	990	442	442	4.2	0.01
2,3-Diethyl-5-methylpyrazine	38	23	21	21	2.1	0.10
2-(sec-Butyl)-3-methoxy-pyrazine	23	14	15	14	0.9	0.06
2-Ethyl-3-methoxy-pyrazine	125	70	52	52	2.9	0.06
2-Methoxy-3-methylpyrazine	309	167	101	101	8.4	0.08

EU: European Union; PNEC_{soil}: predicted no effect concentration for terrestrial environment; PEC_{soil}: predicted environmental concentration for soil; PNEC_{aquatic}: predicted no effect concentration for aquatic compartment.

(a): LC₅₀: the concentration of a test substance which results in a 50% mortality of the test species.

(b): EC₅₀: 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 all eight compounds, the ratio PEC/PNEC for soil and surface water was < 1 (Table 6), indicating that there is no risk for the terrestrial and fresh water compartments at the maximum proposed use level of 0.5 mg/kg.

If used in fish feed at the highest proposed use level of 0.5 mg/kg complete feed in land-based aquaculture systems, none of all 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, 2008b). For sea cages, a dietary concentration of 0.047 mg/kg would ensure that the threshold for the predicted environmental concentration of the additive (parent compound) in sediment (PEC_{sed}) of 10 µg/kg is not exceeded when calculated according to the EFSA guidance (EFSA, 2008b).

3.2.6.1. Conclusions on safety for the environment

The maximum proposed use level of 0.5 mg/kg is unlikely to have detrimental effects on the terrestrial and fresh water compartments for any of the compounds under application. For the marine environment, the safe use level is estimated to be 0.05 mg/kg feed.

3.3. Efficacy

Since all 22 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 2,3-diethylpyrazine [14.005], 2-ethyl-3-methylpyrazine [14.006], 5,6,7,8-tetrahydroquinoxaline [14.015], 2,3,5,6-tetramethylpyrazine [14.018], 2,3,5-trimethylpyrazine [14.019], 2,5-dimethylpyrazine [14.020], 2,6-dimethylpyrazine [14.021], 2-ethylpyrazine [14.022], 2-ethyl-3,5-dimethylpyrazine [14.024], 2,5 or 6-methoxy-3-methylpyrazine [14.025], 2-methylpyrazine [14.027], acetylpyrazine [14.032], 6,7-dihydro-5-methyl-5*H*-cyclopenta(b)pyrazine [14.037], 2-isobutyl-3-methoxy-pyrazine [14.043], 2-acetyl-3-ethylpyrazine [14.049], 2,3-dimethylpyrazine [14.050], 2,3-diethyl-5-methylpyrazine [14.056], 2-(*sec*-butyl)-3-methoxy-pyrazine [14.062], 3,(5- or 6-)-dimethyl-2-ethylpyrazine [14.100], 2-ethyl-3-methoxy-pyrazine [14.112] and 2-methoxy-3-methylpyrazine [14.126] are safe at the proposed maximum dose level (0.5 mg/kg complete feed) as feed for cattle, salmonids and non-food-producing animals, and at the proposed normal use level of 0.1 mg/kg complete feed for pigs and poultry; 5-methylquinoxaline [14.028] is safe only at concentrations below the proposed use levels (0.08 mg/kg complete feed for cattle, salmonids and non-food-producing animals, and 0.05 mg/kg complete feed for pigs and poultry).

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

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

The maximum proposed use level of 0.5 mg/kg is unlikely to have detrimental effects on the terrestrial and fresh water compartments for any of the compounds under application.

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 24 – Pyrazine derivatives for all animal species and categories. July 2010. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
- 2) Chemically defined flavourings from Flavouring Group 24 – Pyrazine derivatives for all animal species and categories. Supplementary information. June 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
- 3) Chemically defined flavourings from Flavouring Group 24 – Pyrazine derivatives for all animal species and categories. Supplementary information. January 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
- 4) Chemically defined flavourings from Flavouring Group 24 – Pyrazine derivatives for all animal species and categories. Supplementary information. September 2016. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
- 5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Chemically Defined Flavourings – from Chemical Group 24 – Pyrazine derivatives.
- 6) Comments from the Member States.

References

Arnot J, Gouin T and Mackay D, 2005. Practical Methods for Estimating Environmental Biodegradation Rates, Report to Environment Canada. CEMN Report No 200503. Canadian Environmental Modelling Network, Trent University, Peterborough, ON, Canada.

- Blevins S, Siegel PB, Blodgett DJ, Ehrich M and Lewis RM, 2012. Liver enzymes in White Leghorns selected for the sheep red blood cell immune response. *Poultry Science*, 91, 322–326.
- Court MH, 2013. Feline drug metabolism and disposition: pharmacokinetic evidence for species differences and molecular mechanisms. *The Veterinary Clinics of North America. Small Animal Practice*, 43, 1039–1054. doi:10.1016/j.cvsm.2013.05.002.
- EFSA (European Food Safety Authority), 2008a. Flavouring Group Evaluation 53 (FGE.53): Consideration of phenethyl alcohol, aldehyde, acid and related acetals and esters evaluated by JECFA (59th meeting) structurally related to phenethyl alcohol, aldehyde, esters and related phenylacetic acid esters evaluated by EFSA in FGE.14 (2005) and one phenoxyethyl ester evaluated in FGE.23 (2006) (Commission Regulation (EC) No 1565/2000 of 18 July 2000) – Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC). *EFSA Journal* 2008;6(5):710, 39 pp. doi:10.2903/j.efsa.2008.710
- EFSA (European Food Safety Authority), 2008b. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. *EFSA Journal* 2008;6(10):842, 28 pp. doi:10.2903/j.efsa.2008.842
- EFSA CEF Panel (Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food), 2011a. Scientific Opinion on the Flavouring Group Evaluation 50, Revision 1 (FGE.50Rev1): Consideration of pyrazine derivatives evaluated by JECFA (57th meeting) structurally related to pyrazine derivatives evaluated by EFSA in FGE.17Rev2 (2010) (Commission Regulation (EC) No 1565/2000 of 18 July 2000). *EFSA Journal* 2011;9(5):1921, 41 pp. doi:10.2903/j.efsa.2011.1921
- EFSA CEF Panel (Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food), 2011b. Scientific Opinion on the Flavouring Group Evaluation 17, Revision 3 (FGE.17Rev3): pyrazine derivatives from chemical group 24. *EFSA Journal* 2011;9(11):2456, 67 pp. doi:10.2903/j.efsa.2011.2456
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for sensory additives. *EFSA Journal* 2012;10(1):2534, 26 pp. doi:10.2903/j.efsa.2012.2534
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for the preparation of dossiers for additives already authorised for use in food. *EFSA Journal* 2012;10(1):2538, 4 pp. doi:10.2903/j.efsa.2012.2538
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance for establishing the safety of additives for the consumer. *EFSA Journal* 2012;10(1):2537, 12 pp. doi:10.2903/j.efsa.2012.2537
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012d. Guidance on studies concerning the safety of use of the additive for users/workers. *EFSA Journal* 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539
- FAO (Food and Agricultural Organization of the United Nations), 2006. FAO JECFA Monographs 1: Combined Compendium of Food Additive Specifications—Joint FAO/WHO Expert Committee on Food Additives—All specifications monographs from the 1st to the 65th meeting (1956–2005). Volume 4. Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications. Food and Agricultural Organization of the United Nations, Rome, Italy. Available online: <http://www.fao.org/docrep/009/a0691e/a0691e00.htm>
- Felsted RL and Bachur NR, 1980. Chapter 13, Ketone reductase. In: Jakoby WB (ed.). *Biochemical Pharmacology and Toxicology. A Series of Monographs. Enzymatic basis of detoxication*, Vol. 1, Academic Press INC, New York, USA. pp. 281–292.
- Fink-Gremmels J, 2008. Implications of hepatic cytochrome P450-related biotransformation processes in veterinary sciences. *European Journal of Pharmacology*, 585, 502–509.
- Gupta RC, 2007. *Veterinary Toxicology. Basic and Clinical Principles*. Elsevier, Amsterdam, the Netherlands.
- Gusson F, Carletti M, Giuliano Albo A, Dacasto M and Nebbia C, 2006. Comparison of hydrolytic and conjugative biotransformations pathways in horse, cattle, pig, broiler chick, rabbit and rat liver subcellular fractions. *Veterinary Research Communications*, 30, 271–283.
- Hainline BE and Rajagopalan KV, 2013. Molybdenum in animal and human health. In: Rose J (ed.). *Trace Elements in Health: A Review of Current Issues*. Butterworths & Co, London, UK, pp. 150–166.
- Hawksworth G and Scheline RR, 1975. Metabolism in the rat of some pyrazine derivatives having flavour importance in foods. *Xenobiotica*, 5, 389–399.
- Ioannides C, 2006. Cytochrome P450 expression in the liver of food-producing animals. *Current Drug Metabolism*, 7, 335–348.
- James MO, 1987. Conjugation of organic pollutants in aquatic species. *Environmental Health Perspectives*, 71, 97–103.
- Morikawa Y, Yamamoto T and Higashino K, 1997. Distribution and pathophysiologic role of molybdenum-containing enzymes. *Histology and Histopathology*, 12, 513–524.
- Müller R and Rappert S, 2010. Pyrazine: occurrence, formation and biodegradation. *Applied Microbiology and Biotechnology*, 85, 1315–1320.
- Nebbia C, Dacasto M, Rossetto Giaccherino A, Giuliano Albo A and Carletti M, 2003. Comparative expression of liver cytochrome P450-dependent monooxygenases in the horse and in other agricultural and laboratory species. *Veterinary Journal*, 165, 53–64.

- Posternak JM, Linder A and Vodoz CA, 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. *Food Cosmetic Toxicology*, 7, 405–407.
- Posternak JM, Dufour JJ, Rogg C and Vodoz CA, 1975. Summaries of toxicological data. Toxicological tests on flavouring matters. II. Pyrazines and other compounds. *Food and Cosmetic Toxicology*, 13, 487–490.
- Watkins JB III and Klaassen CD, 1986. Xenobiotic biotransformation in livestock: comparison to other species commonly used in toxicity testing. *Journal of Animal Science*, 63, 933–942.
- Wheldon GH and Krajceman AJ, 1967. The effect of ten food-flavouring additives administered to rats over a period of thirteen weeks. Huntingdon Research Centre. Unpublished report, 30 June 1967.
- WHO (World Health Organization), 2002a. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 909. Geneva, Switzerland, 5–14 June 2001.
- WHO (World Health Organization), 2002b. Safety Evaluation of Certain Food Additives and Contaminants. Prepared by the Fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva (WHO Food Additives Series, 48).
- Wolf JC and Wolfe MJ, 2005. A brief overview of nonneoplastic hepatic toxicity in fish. *Toxicologic Pathology*, 33, 75–85.

Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CDG	chemically defined group
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
DM	dry matter
DT ₅₀	degradation half-time
EC ₅₀	half-maximal effective concentration
ECOSAR	component program of EPI suite™
EEIG	European Economic Interest Grouping
EPI suite	Estimation Programs Interface (EPI) Suite™
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	the EU Flavour Information System
FL-No	FLAVIS number
GC–MS	gas chromatography–mass spectrometry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
K _{oc}	organic carbon sorption constant
K _{ow}	octanol–water partition coefficient
LC ₅₀	lethal concentration 50
Log K _{ow}	logarithm of octanol–water partition coefficient
NOAEL	no observed adverse effect level
PEC	predicted environmental concentration
PEC _{sed}	predicted environmental concentration of the additive (parent compound) in sediment
PEC _{porewater}	predicted environmental concentration for porewater
PEC _{soil}	predicted environmental concentration for soil
PEC _{surfacewater}	predicted environmental concentration for surface water
PEC _{swaq}	predicted environmental concentration of the additive (parent compound) in surface water
PNEC	predicted no environmental concentration
PNEC _{soil}	predicted no environmental concentration for terrestrial environment

PNEC _{aquatic}	predicted no environmental concentration for aquatic compartment
RTL	retention time locking
SMILES	Simplified Molecular Input Line Entry Specification
TNO	Netherlands Organisation for Applied Scientific Research
TTC	threshold of toxicological concern
UF	uncertainty factor
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Chemically defined flavourings from Chemical group 24 – Pyrazine derivatives

The *Chemically Defined Flavourings - Group 24 (Pyrazine derivatives)*, in this application comprises 22 substances, for which authorisation as feed additives is sought under the category “sensory additives”, functional group 2(b) “flavouring compounds”, according to the classification system of Annex I of Regulation (EC) No 1831/2003.

In the current application submitted according to Article 4(1) and Article 10 (2) of Regulation (EC) No 1831/2003, the authorisation for all species and categories is requested. The flavouring compounds of interest have a purity ranging from 95% to 99%.

Mixtures of flavouring compounds are intended to be incorporated only into *feedingstuffs* or drinking *water*. The Applicant suggested no minimum or maximum levels for the different flavouring compounds in *feedingstuffs*.

For the identification of volatile chemically defined flavouring compounds *CDG24* in the *feed additive*, the Applicant submitted a qualitative multi-analyte gas-chromatography mass-spectrometry (GC-MS) method, using Retention Time Locking (RTL), which allows a close match of retention times on GC-MS. By making an adjustment to the inlet pressure, the retention times can be closely matched to those of a reference chromatogram. It is then possible to screen samples for the presence of target compounds using a mass spectral database of RTL spectra. The Applicant maintained two FLAVOR2 databases/libraries (for retention times and for MS spectra) containing data for more than 409 flavouring compounds. These libraries were provided to the CRL. The Applicant provided the typical chromatogram for the *CDG24* 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 *CDG24* 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 *CDG24* in the *mixture of flavouring compounds*.

Based on the satisfactory experimental evidence provided, the CRL recommends for official control for the qualitative identification in the *feed additive* of the individual (or mixture of) *flavouring compounds* of interest the GC-MS-RTL (Agilent specific) method submitted by the Applicant.

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