SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 8 March 2016

doi: 10.2903/j.efsa.2016.4441

Safety and efficacy of thiazoles, thiophene and thiazoline belonging to chemical group 29 when used as flavourings for all animal species

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

Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of 12 compounds belonging to chemical group 29 (thiazoles, thiophene and thiazoline). They are currently authorised as flavours in food. This opinion concerns 10 compounds from this group. The FEEDAP Panel concludes that 2-isopropyl-4-methylthiazole is safe at the proposed maximum use level of 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. All the other compounds, 4, 5-dihydrothiophen-3(2H)-one, 2-isobutylthiazole, 5-(2-hydroxyethyl)-4-methylthiazole, benzothiazole, 2,4, 5-trimethylthiazole, 2-acetylthiazole, 2-ethyl-4-methylthiazole, 5,6-dihydro-2,4,6-trans(2-methylpropyl) 4H-1,3,5-dithiazine and thiamine hydrochloride are safe at the proposed maximum use level of 0.05 mg/kg feed for all animal species. No safety concern would arise for the consumer from the use of these compounds up to the highest safe 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 concentrations considered safe for the target species are unlikely to have detrimental effects on the terrestrial and fresh water environments. As 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. In the absence of data on the stability in water for drinking, the FEEDAP Panel is unable to conclude on the safety or efficacy of the substances under this mode of delivery.

Keywords: sensory additives, flavourings, thiazoles, thiophene, thiazoline and thienyl derivatives, chemical group 29

Requestor: European Commission

Question number: EFSA-Q-2013-00569

Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, 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.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Feed Flavourings, including Paul Brantom, Birgit Dusemund, Christer Hogstrand, Patrick Van Beelen and Johannes Westendorf, for the support provided to this scientific output.

Suggested citation: FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of thiazoles, thiophene and thiazoline belonging to chemical group 29 when used as flavourings for all animal species. EFSA Journal 2016; 14(6):4441, 16 pp. doi:10.2903/j.efsa.2016.4441

ISSN: 1831-4732

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 substances 5
3.1.2. Stability and homogeneity 7
3.1.3. Conditions of use 7
3.2. Safety 8
3.2.1. Absorption, distribution, metabolism and excretion 8
3.2.2. Toxicological studies 8
3.2.3. Safety for the target species 9
3.2.3.1. Conclusions on safety for the target species 10
3.2.4. Safety for the consumer 10
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 12
3.3. Efficacy 13
4. Conclusions 13
Documentation provided to EFSA 13
References 13
Abbreviations 15
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Thiazoles, Thiophene, Thiazoline and Thienyl Derivatives 16

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7 and in addition, Article 10(2) of that Regulation 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 12 substances (2,4,5-trimethylthiazole, 2-isobutylthiazole, 5-(2-hydroxyethyl)-4-methylthiazole, benzothiazole, 4-methyl-5-vinylthiazole, 2,4,5-trimethylthiazole, 2-acetylthiazole, 3-acetyl-2,5-dimethylthiophene, 2-isopropyl-4-methylthiazole, 2-ethyl-4-methylthiazole, 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine and thiamine hydrochloride) belonging to chemical group (CG) 29, when used as a feed additive for all animal species (category: sensory additives; functional group: flavourings). CG 29 for flavouring substances is defined in Commission Regulation (EC) No 1565/2000[3] as 'thiazoles, thiophene, thiazoline and thienyl derivatives.' During the course of the assessment, this application was split and the present opinion covers 10 out of the 12 substances under application (see Section 1.2).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). 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 18 November 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 2,4,5-trimethylthiazole, 2-isobutylthiazole, 5-(2-hydroxyethyl)-4-methylthiazole, benzothiazole, 2,4,5-trimethylthiazole, 2-acetylthiazole, 2-isopropyl-4-methylthiazole, 2-ethyl-4-methylthiazole, 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine and thiamine hydrochloride, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The 12 compounds have been previously assessed by Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 2000, 2002). JECFA concluded that all 12 flavouring substances evaluated were of no safety concern when used at current levels of estimated intake. No acceptable daily intake values were specified.

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (EFSA, 2008a; EFSA CEF Panel 2013a,b) agreed with JECFA conclusions for 10 of the 12 compounds, but raised concerns for genotoxicity for 4-methyl-5-vinylthiazole [EU Flavour Information System (FLAVIS) number 15.018] and 3-acetyl-2,5-dimethylthiophene [15.024]. 3-Acetyl-2,5-dimethylthiophene [15.024] was confirmed to be genotoxic and excluded from food use (EFSA CEF Panel, 2013b). Subsequently, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on this compound also concluding that its use in feed was not safe (EFSA FEEDAP Panel, 2013). The assessment of 4-methyl-5-vinylthiazole [15.018] has yet to be completed and consequently the FEEDAP Panel will also not proceed with an assessment of this compound until the issue of genotoxicity has been resolved.

[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] Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG), Avenue Louise 130A, B-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.

The current assessment concerns the remaining 10 compounds, all of which are currently listed in the European Union database of flavouring substances[4] and in the European Union Register of Feed Additives, respectively, and thus authorised for use in food and feed in the European Union. They have not been previously assessed by EFSA as feed additives.

Regulation (EC) No 429/2008[5] allows substances already approved for use in human food to be assessed with a more limited procedure than for other feed additives. However, the use of this procedure is always subject to the condition that food safety assessment is relevant to the use in feed.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[6] in support of the authorisation request for the use of thiazoles, thiophene, thiazoline and thienyl derivatives as feed additives. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008 and the applicable EFSA guidance documents.

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

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the thiazoles, thiophene, thiazoline and thienyl derivatives in animal feed. The Executive Summary of the EURL report can be found in Annex A.[7]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of compounds belonging to CG 29 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 substances

The molecular structures of the 10 additives under assessment are shown in Figure 1 and their physico-chemical characteristics in Table 1.

[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] 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.

[6] FEED dossier reference: FAD-2010-0410.

[7] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0116.pdf

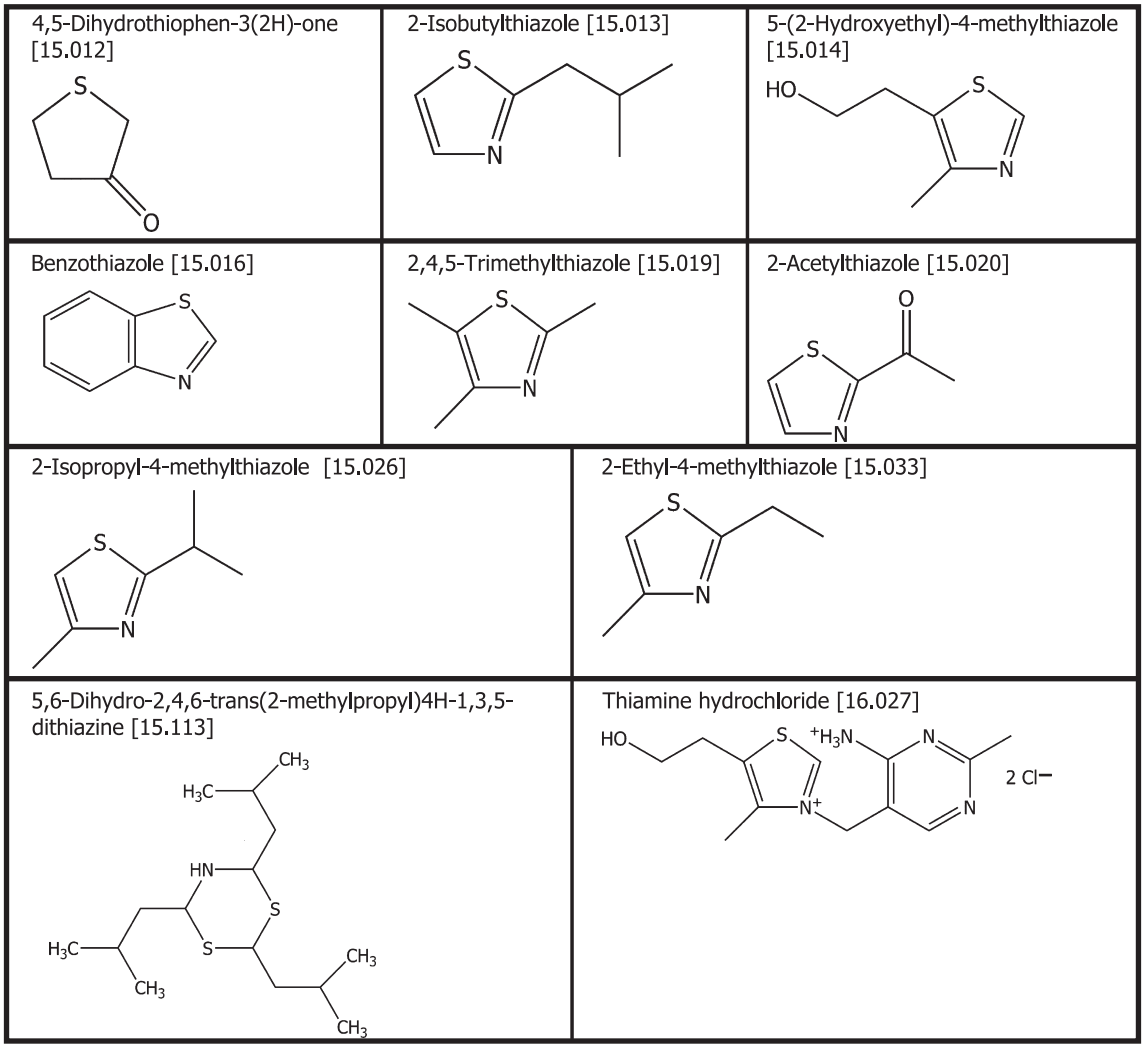

Figure 1: Molecular structures and [FLAVIS numbers] of the 10 flavouring compounds under assessment

Table 1: Chemical Abstracts Service (CAS) and FLAVIS numbers and some characteristics of the 10 flavouring compounds under assessment

EU Register name	CAS No	FLAVIS No	Molecular formula	Molecular weight	Physical state	Log K_{ow} [(a)]
4,5-Dihydrothiophen-3(2H)-one	1003-04-9	15.012	C_4H_6OS	102.15	Liquid	−0.29
2-Isobutylthiazole	18640-74-9	15.013	$C_7H_{11}NS$	141.24	Liquid	2.51
5-(2-Hydroxyethyl)-4-methylthiazole	137-00-8	15.014	C_6H_9ONS	143.21	Liquid	1.11
Benzothiazole	95-16-9	15.016	C_7H_5NS	135.19	Liquid	2.01
2,4,5-Trimethylthiazole	13623-11-5	15.019	C_6H_9NS	127.21	Liquid	2.02
2-Acetylthiazole	24295-03-2	15.020	C_5H_5ONS	127.17	Liquid	0.74
2-Isopropyl-4-methylthiazole	15679-13-7	15.026	$C_7H_{11}NS$	141.24	Liquid	2.44
2-Ethyl-4-methylthiazole	15679-12-6	15.033	C_6H_9NS	127.21	Liquid	2.09
5,6-Dihydro-2,4,6-trans (2-methylpropyl) 4H-1,3,5-dithiazine	74595-94-1	15.113	$C_{15}H_{31}NS_2$	289.55	Solid	6.08
Thiamine hydrochloride	67-03-8	16.027	$C_{12}H_{18}ON_4S$	337.27	Solid	–

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

All of the compounds under consideration are produced by chemical synthesis and typical routes of synthesis are described for each compound.[8]

Data were provided on the batch to batch variation in five batches of each additive except for benzothiazole [15.016], for which only two batches were available due to the low use volume (< 1 kg/year).[9] The content of the active substance exceeded the JECFA specifications for all compounds except 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine [15.113] (Table 2).

Table 2: Identity of the substances and data on purity

EU Register name	FLAVIS No	JECFA specification minimum %[(a)]	Assay %	
			Average	Range
4,5-Dihydrothiophen-3(2H)-one	15.012	97	99.8	99.7–100
2-Isobutylthiazole	15.013	96	99.5	99.2–99.7
5-(2-Hydroxyethyl)-4-methylthiazole	15.014	96	99.9	99.9–100
Benzothiazole	15.016	96	98.0[(b)]	98.0–98.0
2,4,5-Trimethylthiazole	15.019	97	99.7	99.5–99.9
2-Acetylthiazole	15.020	97	99.7	99.16–100
2-Isopropyl-4-methylthiazole	15.026	96	99.7	99.5–100
2-Ethyl-4-methylthiazole	15.033	97	99.8	99.6–100
5,6-Dihydro-2,4,6-trans(2-methylpropyl) 4H-1,3,5-dithiazine	15.113	95[(c)]	88.2	87–89.2
Thiamine hydrochloride	16.027	98	99.8	99.2–100.4

EU: European Union; Flavis number: EU Flavour Information System numbers; JECFA: The Joint FAO/WHO Expert Committee on Food Additives.
(a): FAO, 2006.
(b): Two batches, use of the product is 1 kg/year or less.
(c): According to JECFA: Min. assay value is '95% (mixture of 3 stereoisomers).' Mixture of diastereoisomers, each of them racemic (EFSA CEF Panel, 2013b). The applicant uses specifications of 87%.

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 and homogeneity

The minimum shelf-life of the compounds under assessment ranges from 12 to 36 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.

Although no data are required for the stability of flavouring additives in premixes and feed, their use in water for drinking introduces other issues relating to product stability, such as degradation due to microbial activity. The FEEDAP Panel notes that six out of the 10 compounds in CG 29 have a low water solubility (Log K_{ow} > 2), which makes it difficult to assess the safety in water for drinking. Considering this, and the absence of data on the short-term stability and the capacity to homogeneously distribute in water for drinking, the FEEDAP Panel is not in the position to conclude on the use of the additives in water for drinking.

3.1.3. Conditions of use

The applicant proposes the use of all of the 10 compounds in feed or water for drinking for all animal species without withdrawal. For 2-isopropyl-4-methylthiazole [15.026], the applicant proposes a normal use level of 0.1 mg/kg feed and a high use level of 0.5 mg/kg. For the remaining nine additives, the applicant proposes a normal use level of 0.01 mg/kg feed and a high use level of 0.05 mg/kg feed. No proposals are made for the dose to be used in water for drinking.

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

3.2. Safety

The assessment of safety is based on the high use levels proposed by the applicant (0.5 mg/kg for 2-isopropyl-4-methylthiazole [15.026] and 0.05 mg/kg complete feed for the remaining compounds).

3.2.1. Absorption, distribution, metabolism and excretion

Little information is available regarding the absorption and distribution of compounds belonging to CG 29, following oral ingestion. Owing to their lipophilicity, the FEEDAP Panel considers that most of these compounds are likely to be fully absorbed from the intestine of the target animals and distributed within their bodies.

Cyclic sulphides containing oxidised carbon, like 4,5-dihydrothiophen-3(2H)-one [15.012], are predicted to be metabolised by extensive S-oxidation and conjugation of alcohol groups with glucuronic acid or sulfate (WHO, 2000).

Thiazole and its derivatives, namely 2-isobutylthiazole [15.013], benzothiazole [15.016], 2,4,5-trimethylthiazole [15.019], 2-acetylthiazole [15.020], 2-isopropyl-4-methylthiazole [15.026] and 2-ethyl-4-methylthiazole [15.033], are metabolised primarily by side-chain oxidation or oxidation of the ring sulfur or nitrogen atoms (Rance 1989, as quoted in WHO, 2003). The major metabolites are then readily excreted in the urine either free or as glutathione conjugates (WHO, 2003). However, other routes of metabolism, involving ring cleavage, are also possible. Ring C-oxidation may be accompanied by heterocyclic ring cleavage for some thiazole derivatives resulting in the formation of alpha-diketone and thioamide intermediates (EFSA CEF Panel, 2012a,b).

Benzothiazole [15.016] is primarily metabolised in guinea pigs by thiazole ring cleavage. A 30 mg/kg bw dose administered by intraperitoneal injection is metabolised to free or conjugated forms of o-aminophenyl methyl sulfide, o-aminophenyl methyl sulfoxide, and o-aminophenyl methyl sulfone. Small amounts of the N-hydroxyderivatives of the sulfoxide and sulfone were also detected in urine (Wilson et al., 1991).

The metabolism of thiamine [16.027] (vitamin B_1) is well recognised and has been previously described in a number of FEEDAP opinions (EFSA FEEDAP Panel, 2011a,b,c).

Derivatives of thiazine and dithiazine, like 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine [15.113], are expected to be metabolised primarily via side-chain oxidation and by ring S- and N-oxidation (EFSA CEF Panel, 2012a).

No metabolic studies in target species could be found for CG 29 compounds. For thiazole and thiophene compounds, it is assumed that the fate in the target species is similar to that occurring in experimental animals. The enzymes involved in the biotransformation pathways of CG 29 compounds have been detected in many species, including mammals, birds and fish, and are assumed to be present in all the target species. The enzymes include cytochrome P450 monooxygenase families (Nebbia et al., 2003; Ioannides, 2006), glucuronide-, sulfate- and glutathione transferases (Watkins and Klaassen, 1986; Gusson et al., 2006). Thus, it is expected that the target species are able to metabolise these compounds and no appreciable residues are expected to remain in the food products for consumers.

3.2.2. Toxicological studies

Toxicological data (sub-chronic, repeated-dose studies, with multiple doses tested) could be found only for 2-acetylthiazole [15.020].

A 90-day feeding study with 2-acetylthiazole [15.020] was performed in rats at doses of 100, 1,000 and 10,000 mg/kg feed, corresponding to 5, 50 and 500 mg/kg bw per day (Wheldon et al., 1970). No signs of toxicity were observed at the two lower doses. At the highest dose tested, body weight changes, due to depressed food consumption with concomitant reduction of growth and marginally increased steatosis of the liver were observed. No haematological disturbances occurred. From this study a no observed adverse effect level (NOAEL) of 50 mg/kg bw per day can be derived.

Secondary references referred to a repeated dose toxicity study (90 days, only one dose tested) in rat with benzothiazole [15.016] in which a NOAEL of 5.1 mg/kg bw per day was identified (Morgareidge, 1971, unpublished). However, the study report was not available and the NOAEL could not be confirmed.

3.2.3. Safety for the target species

The first approach to the safety assessment for target species takes account of the intended use levels in animal feed relative to the maximum reported exposure of humans on the basis of the metabolic body weight (kg $bw^{0.75}$). Human exposure in the EU to the individual compounds ranges from 0.2 to 1,200 μg/person per day (EFSA CEF Panel, 2013a,b). This corresponds to 0.01–55.7 μg/$kg^{0.75}$ per day. These exposure levels are considered safe for humans. Table 3 summarises the result of the comparison with human exposure for representative target animals.

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

EU Register name	Use level in feed (mg/kg)	Human exposure (μg/kg $bw^{0.75}$ per day)[(a)]	Target animal exposure μg/kg $bw^{0.75}$/day		
			Salmon	Piglet	Dairy cow
4,5-Dihydrothiophen-3(2H)-one	0.05	0.02	1.18	5.26	7.77
2-Isobutylthiazole	0.05	0.02	1.18	5.26	7.77
5-(2-Hydroxyethyl)-4-methylthiazole	0.05	17.6	1.18	5.26	7.77
Benzothiazole	0.05	0.01	1.18	5.26	7.77
2,4,5-Trimethylthiazole	0.05	0.01	1.18	5.26	7.77
2-Acetylthiazole	0.05	0.46	1.18	5.26	7.77
2-Isopropyl-4-methylthiazole	0.5	0.46	11.8	52.6	77.7
2-Ethyl-4-methylthiazole	0.05	0.05	1.18	5.26	7.77
5,6-Dihydro-2,4,6-trans(2-methylpropyl) 4H-1,3,5-dithiazine	0.05	0.12	1.18	5.26	7.77
Thiamine hydrochloride	0.05	55.7	1.18	5.26	7.77

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

Table 3 shows that for all compounds, except 5-(2-hydroxyethyl)-4-methylthiazole [15.014] and thiamine hydrochloride [16.027], the intake by the target animals greatly exceeds that of humans, resulting from use in food. Thiamine is a vitamin for humans and animals (vitamin B_1), which is devoid of toxicity at oral application in different animal species even at extreme doses (Lang, 1979). Vitamin B_1 supplementation (ranging from 1 mg/kg in pigs and poultry up to 15 mg/kg in fish) is safe for all animal species with a wide margin of safety of about 1,000 compared to the requirements/ recommendations (EFSA FEEDAP Panel, 2011a,b,c). The FEEDAP Panel concludes that 5-(2-hydroxyethyl)-4-methylthiazole and thiamine hydrochloride are safe for the target species at the proposed maximum dose level (0.05 mg/kg feed).

Safety for the target species at the feed concentration applied for the remaining eight compounds cannot be derived from the risk assessment for food use. As an alternative, the maximum feed concentration which can be considered safe for the target animals can be derived from the lowest NOAEL if suitable data are available.

Toxicological data derived from a sub-chronic, repeated-dose study were available for 2-acetylthiazole [15.020] (see Section 3.2.2). Applying an uncertainty factor (UF) of 100 to the NOAEL, the maximum safe intake for the target species was derived following the EFSA Guidance for sensory additives (EFSA FEEDAP Panel, 2012a), and thus the maximum safe feed concentration was calculated (Table 4).

Table 4: Maximum safe concentration in feed for different target animals for 2-acetylthiazole

Target animal	Default values		Maximum safe intake/feed concentration	
	Body weight (kg)	Feed intake (g/day)[(a)]	Intake (mg/day)	Concentration (mg/kg feed)[(b)]
Salmonids	2	40	1	25
Veal calves (milk replacer)	100	2,000	50	25
Cattle for fattening	400	8,000	200	22
Dairy cows	650	20,000	325	14

Target animal	Default values		Maximum safe intake/feed concentration	
	Body weight (kg)	Feed intake (g/day)[a]	Intake (mg/day)	Concentration (mg/kg feed)[b]
Piglets	20	1,000	10	10
Pigs for fattening	100	3,000	50	17
Sows	200	6,000	100	17
Chickens for fattening	2	120	1	8
Laying hens	2	120	1	8
Turkeys for fattening	12	400	6	15
Dogs	15	250	7.5	26
Cats	3	60	1.5	22

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

As individual reliable NOAELs could not be found for the remaining seven compounds, the threshold of toxicological concern (TTC) approach was followed to derive the maximum safe feed concentration (EFSA FEEDAP Panel, 2012a).

For six Cramer class II compounds, i.e. 4,5-dihydrothiophen-3(2H)-one [15.012], 2-isobutylthiazole [15.013], 2,4,5-trimethylthiazole [15.019], 2-isopropyl-4-methylthiazole [15.026], 2-ethyl-4-methylthiazole [15.033] and 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine [15.113], the calculated safe use level 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. The FEEDAP Panel notes that the purity of 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine [15.113] for feed use is lower than in the JECFA specification (88 vs 95%). However, considering the intended use level, this difference is not considered of concern.

For benzothiazole [15.016], a Cramer class III compound, the safe use level is 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.

3.2.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that:

- 2-isopropyl-4-methylthiazole [15.026] is safe at the proposed maximum use level of 0.5 mg/kg complete feed for cattle, salmonids and non-food producing animals and at the proposed use level of 0.1 mg/kg complete feed for pigs and poultry;
- 4,5-dihydrothiophen-3(2H)-one [15.012], 2-isobutylthiazole [15.013], 5-(2-hydroxyethyl)-4-methylthiazole [15.014], benzothiazole [15.016], 2,4,5-trimethylthiazole [15.019], 2-acetylthiazole [15.020] 2-ethyl-4-methylthiazole [15.033], 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine [15.113] and thiamine hydrochloride [16.027] are safe for all target species at the proposed maximum use level (0.05 mg/kg complete feed).

3.2.4. Safety for the consumer

The safety for the consumer of the 10 compounds used as food flavours has been already assessed by JECFA (WHO, 2000, 2002) and EFSA (EFSA, 2008a; EFSA CEF Panel, 2013a,b). All compounds are currently authorised in the EU as food flavourings without limitations.[4]

Given the low use levels of CG 29 compounds to be applied in feed, and the expected extensive metabolism and excretion in target animals (see Section 3.2.1), the FEEDAP Panel considers that the possible residues in food derived from animals fed 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 10 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,[10] 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 five substances, namely 2-isobutylthiazole [15.013], 5-(2-hydroxyethyl)-4-methylthiazole [15.014], benzothiazole [15.016], 2,4,5-tri-methylthiazole [15.019] and 2-acetylthiazole [15.020], which occur in the environment at levels above the application rate of 0.05 mg/kg feed. (Data taken from the Netherlands Organisation for Applied Scientific Research (TNO) database Volatile Compounds in Food *ver.* 14.1; Burdock, 2009).[11]

Thiamine hydrochloride [16.027] is a vitamin for all animals including humans which occurs naturally in their food plants. Therefore, no environmental risk is foreseen for this compound.

The other four compounds, (4,5-dihydrothiophen-3(2H)-one [15.012], 2-isopropyl-4-methylthiazole [15.026], 2-ethyl-4-methylthiazole [15.033] and 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine [15.113]), could not be shown to occur in the environment at levels above the application rate of 0.5 (for 2-isopropyl-4-methylthiazole) and 0.05 mg/kg feed for the remaining three compounds. These substances are therefore assessed in a predicted environmental concentration (PEC) calculation for soil (PEC_{soil}) arising from the application rate. The calculations performed according to the EFSA guidance (2008b) using the most conservative value obtained (lamb manure) are shown in Table 5.

Table 5: PEC values of the flavourings of CG 29 under assessment

EU Register name	CAS No.	Dose mg/kg	PEC_{soil} (μg/kg)	$PEC_{porewater}$ (μg/L)
4,5-Dihydrothiophen-3(2H)-one	1003-04-9	0.05	1	3.7
2-Isopropyl-4-methylthiazole	15679-13-7	0.5	11	1.0
2-Ethyl-4-methyl-thiazole	15679-12-6	0.05	1	0.2
5,6-Dihydro-2,4,6-trans(2-methylpropyl) 4H-1,3,5-dithiazine	74595-94-1	0.05	1	0.0017

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

The value for 2-isopropyl-4-methylthiazole [15.012] is slightly above the threshold of 10 μg/kg (EFSA, 2008b). The $PEC_{porewater}$, however, is dependent on the sorption, which is different for each compound. For these calculations, the substance-dependent constants organic carbon sorption constant (K_{oc}), molecular weight, vapour pressure and solubility are needed. These were estimated from the Simplified Molecular Input Line Entry Specification (SMILES) notation of the chemical structure using EPIWEB 4.1 (Table 6).[12] This program was also used to derive the SMILES notation from the CAS numbers. The K_{oc} value derived from the first-order molecular connectivity index was used, as recommended by the EPIWEB program.

The half-life (DT_{50}) was calculated using BioWin3 (Ultimate Survey Model), which gives a rating number. This rating number r was translated into a half-life using the formula by Arnot et al. (2005):

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

This is the general regression used to derive estimates of aerobic environmental biodegradation half-lives from BioWin3 model output.

Three substances in Table 5 have a $PEC_{porewater}$ above 0.1 μg/L, one of them (2-isopropyl-4-methylthiazole) has also a PEC_{soil} above 10 μg/kg. Therefore, these three substances are subjected to phase II risk assessment.

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

Table 6: Physico-chemical properties predicted by EPIWEB 4.1

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)
4,5-Dihydrothiophen-3(2H)-one	1003-04-9	9	102.15	215	192,000	10
2-Isopropyl-4-methylthiazole	15679-13-7	16	141.23	48.6	229	614
2-Ethyl 4-methylthiazole	15679-12-6	14	127.21	79.1	592	393
5,6-Dihydro-2,4,6-trans (2-methylpropyl)4H-1,3, 5-dithiazine	74595-94-1	23	289.54	0.002	2.18	34,198

EU: European Union; CAS No: Chemical Abstracts Service.
(a): DT_{50}, half-life of the additive (by BioWin 3).
(b): K_{oc}, organic carbon sorption constant.

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

The ratio PEC/PNEC was < 1 (Table 7) for soil and surface water for all compounds, indicating that there is no risk to the environment at the maximum proposed use levels.

Table 7: Phase II environmental risk assessment of soil and aquatic compartment for terrestrial farm animals (Exposure and effect data were modelled using EPIWEB 4.1 and ECOSAR 1.11)

EU Register name Soil	LC_{50}[(a)] Earthworm (mg/kg)			$PNEC_{soil}$ (µg/kg)	PEC_{soil} (µg/kg)	PEC/ PNEC
4,5-Dihydrothiophen-3(2H)-one	305			305	1	0.003
2-Isopropyl-4-methylthiazole	194			194	11	0.12
2-Ethyl 4-methylthiazole	193			193	1	0.05
Aquatic	**LC_{50} Fish (mg/L)**	**LC_{50} Daphnia (mg/L)**	**EC_{50}[(b)] Algae (mg/L)**	**$PNEC_{aquatic}$ (µg/L)**	**$PEC_{surfacewater}$ (µg/L)**	**PEC/ PNEC**
4,5-Dihydrothiophen-3(2H)-one	9,001	4,148	1,303	1,303	1.24	0.0008
2-Isopropyl-4-methylthiazole	15	9	10	9	0.32	0.036
2-Ethyl 4-methylthiazole	32	19	18	18	0.05	0.003

EU: European Union.
(a): LC_{50}, the concentration of a test substance which results in a 50% mortality of the test species.
(b): EC_{50}, the concentration of a test substance which results in 50% of the test animals being adversely affected (i.e. both mortality and sublethal effects).

The use of all additives in fish feed in land-based aquaculture systems does not give a predicted environmental concentration of the additive (parent compound) in surface water (PEC_{swaq}) above the trigger value of 0.1 µg/L when calculated according to the guidance. For sea cages, a safe dose of 0.047 mg/kg feed was calculated according to the EFSA guidance (EFSA, 2008a,b). This dose would give a sediment concentration of 10 µg/kg which is the threshold level of no concern.

3.2.6.1. Conclusions on safety for the environment

The concentrations considered safe for the target species (see Section 3.1.1) are unlikely to have detrimental effects on the terrestrial and fresh water environments. For the marine environment, the safe use level is estimated to be 0.05 mg/kg feed.

3.3. Efficacy

As the 10 compounds are used in food as flavourings,[4] 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-isopropyl-4-methylthiazole [15.026] is safe at the proposed maximum use level of 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. All the other compounds, 4,5-dihydrothiophen-3(2H)-one [15.012], 2-isobutylthiazole [15.013], 5-(2-hydroxyethyl)-4-methylthiazole [15.014], benzothiazole [15.016], 2,4,5-trimethylthiazole [15.019], 2-acetylthiazole [15.020], 2-ethyl-4-methylthiazole [15.033], 5,6-dihydro-2,4,6-trans(2-methylpropyl)4H-1,3,5-dithiazine [15.113] and thiamine hydrochloride [16.027] are safe at the proposed use level of 0.05 mg/kg feed for all animal species.

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

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

The concentrations considered safe for the target species are unlikely to have detrimental effects on the terrestrial and fresh water environments.

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

However, in the absence of data on the proposed concentration and the stability in water for drinking, the FEEDAP Panel is unable to conclude on the safety or efficacy of the substances under this mode of delivery.

Documentation provided to EFSA

1) Chemically Defined Group 29 – Thiazoles, thiophene, thiazoline and thienyl derivatives. September 2010. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
2) Chemically Defined Group 29 – Thiazoles, thiophene, thiazoline and thienyl derivatives. Supplementary information. July 2011. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
3) Chemically Defined Group 29 – Thiazoles, thiophene, thiazoline and thienyl derivatives. Supplementary information. April 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).
4) Chemically Defined Group 29 – Thiazoles, thiophene, thiazoline and thienyl derivatives. Supplementary information. July 2012. 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 Group 29 Thiazoles, thiophene, thiazoline and thienyl derivatives.
6) Comments from 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.

Burdock GA, 2009. *Fenaroli's Handbook of Flavor Ingredients*. Sixth Edition. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.

EFSA (European Food Safety Authority), 2008a. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on the Flavouring Group Evaluation 76, (FGE.76)1 – consideration of sulphur-containing heterocyclic compounds evaluated by JECFA (59th meeting) structurally related to thiazoles, thiophene, thiazoline and thienyl derivatives from chemical group 29, miscellaneous substances from chemical group 30 evaluated by EFSA in FGE.21. EFSA Journal 2008;6(11):875, 36 pp. doi:10.2903/j.efsa.2008.875

EFSA (European Food Safety Authority), 2008b. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed 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 (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012a. Scientific Opinion on Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3): thiazoles, thiophenes, thiazoline and thienyl derivatives from chemical groups 29 and 30. EFSA Journal 2012;10(2):2457, 94 pp. doi:10.2903/j.efsa.2012.2457

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012b. Scientific Opinion on Flavouring Group Evaluation 08, Revision 5 (FGE.08Rev5): aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical groups 20 and 30. EFSA Journal 2012;10(7):2837, 154 pp. doi:10.2903/j.efsa.2012.2837

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013a. Scientific Opinion on Flavouring Group Evaluation 224 (FGE.224): consideration of genotoxic potential for two a,ß-unsaturated thiophenes from subgroup 5.2 of FGE.19 by EFSA. EFSA Journal 2013;11(2):3093, 18 pp. doi:10.2903/j.efsa.2013.3093

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013b. Scientific Opinion on Flavouring Group Evaluation 76, Revision 1 (FGE.76Rev1): consideration of sulphur-containing heterocyclic compounds evaluated by JECFA (59th meeting) structurally related to thiazoles, thiophene, thiazoline and thienyl derivatives from chemical group 29 and miscellaneous substances from chemical group 30 evaluated by EFSA in FGE.21Rev3. EFSA Journal 2013;11(11):3455, 52 pp. doi:10.2903/j.efsa.2013.3455

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011a. Scientific Opinion on the safety and efficacy of vitamin B_1 (thiamine mononitrate and thiamine hydrochloride) as a feed additive for all animal species based on a dossier submitted by Lohmann Animal Health. EFSA Journal 2011;9(11):2411, 17 pp. 10.2903/j.efsa.2011.2411

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011b. Scientific Opinion on the safety and efficacy of vitamin B_1 (thiamine mononitrate and thiamine hydrochloride) as a feed additive for all animal species based on a dossier submitted by DSM Nutritional Products. EFSA Journal 2011;9(11):2413, 17 pp. doi:10.2903/j.efsa.2011.2413

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011c. Scientific Opinion on the safety and efficacy of vitamin B_1 (thiamine mononitrate) as a feed additive for all animal species based on a dossier submitted by VITAC EEIG. EFSA Journal 2011;9(11):2412, 14 pp. doi:10.2903/j.efsa.2011.2412

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

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of thiazoles, thiophene, thiazoline and thienyl derivatives (chemical group 29): 3-acetyl-2,5-dimethylthiophene when used as a flavouring for all animal species. EFSA Journal 2013;11(8):3323, 14 pp. doi:10.2903/j.efsa.2013.3323

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

Gusson F, Carletti M, Giuliano Albo A, Dacasto M and Nebbia C, 2006. Comparison of hydrolitic and conjugative biotransformations pathways in horse, cattle, pig, broiler chick, rabbit and rat liver subcellular fractions. Veterinary Research Communications, 30, 271–283.

Ioannides C, 2006. Cytochrome P450 expression in the liver of food-producing animals. Current Drug Metabolism, 7, 335–348.

Lang K, 1979. *Biochemie der Ernährung*. Dietrich Steinkopff, Verlag, Darmstadt. pp. 470–486.

Morgareidge K, 1971. 90-Day feeding study with benzothiazole in rats. Food and Drug Research Laboratories, Inc. Lab. no. 0246. February 16, 1971. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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. The Veterinary Journal, 165, 53–64.

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, Amyes SJ, Street AE, Hague PH and Mawdesley-Thomas LE, 1970. Toxicity of Wa4295, Sa 927, SH3048, and Wa 3328 in dietary administration to rats over a period of 13 weeks. Unpublished report from Huntingdon Research Centre, United Kingdom. Submitted to WHO by Flavor and Extract Manufacturers' Association of the United States.

WHO (World Health Organization), 2000. Evaluation of certain food additives. Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 896. Roma, 1-10 June 1999.

WHO (World Health Organization), 2002. Evaluation of certain food additives. Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 913. Geneva, 4-14 June 2002.

WHO (World Health Organization), 2003. Safety evaluation of certain food additives. Prepared by the fifty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva (WHO Food Additives Series, FAS 50).

Wilson JE, Chissick H, Fowler AM, Frearson FJ, Gittins M and Swinbourne FJ, 1991. Metabolism of benzothiazole. I. Identification of ring-cleavage products. Xenobiotica, 21, 1179–1183.

Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CDG	chemically defined group
DM	dry matter
DT_{50}	degradation half-time
EC	European Commission
EC_{50}	half-maximal effective concentration
ECOSAR	component program of EPI suite™
EPI suite	Estimation Programs Interface (EPI) Suite™
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FEEDAP Panel	EFSA 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_{swaq}	predicted environmental concentration of the additive (parent compound) in surface water
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 Thiazoles, Thiophene, Thiazoline and Thienyl Derivatives A

The *Chemically Defined Flavourings – Group 29* (*Thiazoles, thiophene, thiazoline and thienyl derivatives*), in this application comprises twelve 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 96% to 98% and 83% for 5,6-Dihydro-2,4,6-trans (2-methylpropyl)4H-1,3,5-dithiazine.

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* or *water*.

For the identification of volatile chemically defined flavouring compounds *CDG 29* in the *feed additive,* the Applicant submitted a qualitative multi-analyte gas-chromatography mass-spectrometry (GC-MS) method, using Retention Time Locking (RTL), which allows a close match of retention times on GC-MS. By making an adjustment to the inlet pressure, the retention times can be closely matched to those of a reference chromatogram. It is then possible to screen samples for the presence of target compounds using a mass spectral database of RTL spectra. The Applicant maintained two FLAVOR2 databases/libraries (for retention times and for MS spectra) containing data for more than 409 flavouring compounds. These libraries were provided to the EURL. The Applicant provided the typical chromatogram for the *CDG 29* 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 *CDG 29* 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 *CDG 29* in the *mixture of flavouring compounds.*

Based on the satisfactory experimental evidence provided, the EURL recommends for official control for the qualitative identification in the *feed additive* of the individual (or mixture of) *flavouring compounds* of interest listed in Table 1 the GC-MS-RTL (Agilent specific) method submitted by the Applicant.

For the determination of *thiamine hydrochloride* in the *feed additive*, the Applicant submitted the US Pharmacopoeia method (USP 28-NF 23) based on reverse phase liquid chromatography with UV detection. The EURL recommends instead for official control the European Pharmacopoeia (Ph. Eur. 6.0, method 01/2008:0303), based on High-Performance Liquid Chromatography (HPLC) for the determination *thiamine hydrochloride* in the *feed additive* – consistent with the previous EURL opinions presented for FAD-2010–0040 and FAD-2010–0052.

The EURL considers this method suitable to be used within the frame of official control.

As no experimental data were provided by the Applicant for the identification of the *active substance(s)* in *feedingstuffs* and *water*, no methods could be evaluated. Therefore the EURL is unable to recommend a method for the official control to identify the *active substance(s)* of interest listed in Table 1 n *feedingstuffs* or *water*.

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